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(54) Title: NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

(57) Abstract

Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

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DESCRIPTION

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NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

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This invention was made with government support under USAID Grant No. LAG-1328-G-00-3030-00. The government has certain rights in this invention.

Technical Field

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This invention relates to nucleic acid vaccines for rickettsial diseases of animals, including humans.

Background of the Invention

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The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus, *Rickettsia prowazekii*, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, *e.g.*, *Rickettsia rickettsii* and *Rickettsia conorii*, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe *Ehrlichiae* have been described. *Ehrlichiae* infect leukocytes and endothelial cells of many different mammalian species, some of them causing serious human and veterinary diseases. Over 400 cases of human ehrlichiosis, including some fatalities, caused by *Ehrlichia chaffeensis* have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

Heartwater is another infectious disease caused by a rickettsial pathogen, namely Cowdria ruminantium, and is transmitted by ticks of the genus Amblyomma. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America.

In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenicol treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] *J. Infect. Dis.* 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant *R. conorii* protein were partially protected even against *R. rickettsii* (Vishwanath, S., G. McDonald, N. Watkins [1990] Infect. Immun. 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes. *e.g.*, protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] Advances in Vet. Sci. and Comp. Med. 27:427-480; Du Plessis, Plessis, J.L. [1970] Onderstepoort J. Vet. Res. 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against *R. prowazekii* and *R. rickettsii*. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective," In *Rickettsia and Rickettsial Diseases*, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood. This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs

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needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinates. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

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A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsia. The genes encoding the antigens and that can be employed to recombinantly produce those antigen have also been identified and sequenced. Certain protective antigens identified for *R. rickettsii*, *R. conorii*, and *R. prowazekii* (e.g., rOmpA and rOmpB) are large (>100 kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

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Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue, WHO meeting on nucleic acid vaccines [1994] *Vaccine* 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CTL). T-helper 1, and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donelly, S. Parker *et al.* [1993] *Science* 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specificCTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the *Plasmodium voelii* circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] *Proc. Natl. Acad. Sci. USA* 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox. G., T. Zamb, L. Babiuk [1993] *J. Virol.* 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has

WO 00/65063

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not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

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Brief Summary of the Invention

Disclosed and claimed here are novel vaccines for conferring immunity to rickettsia infection, including *Cowdria ruminantium* causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to confer immunity in a susceptible host. Also disclosed are novel materials and methods for diagnosing infections by *Ehrlichia* in humans or animals.

vaccine containing the major antigenic protein 1 gene (MAP1) or the major antigenic protein 2 gene (MAP2) of rickettsial pathogens. In one embodiment, the nucleic acid vaccines can be driven by the human cytomegalovirus(HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention.

immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in *in vitro* lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia. Accordingly, the subject

One aspect of the subject invention concerns a nucleic acid, e.g., DNA or mRNA.

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The subject invention further concerns the genes designated Cowdria ruminantium map 2. Cowdria ruminantium Thworf3. Cowdria ruminantium 4hworf1. Cowdria ruminantium 18hworf1, and Cowdria ruminantium 3gdorf3 and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

invention concerns the discovery that DNA vaccines can induce protective immunity against

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In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine.

Brief Description of the Drawings

Figures 1A-1C show a comparison of the amino acid sequences from alignment of the three rickettsial proteins, namely, $Cowdria\ ruminantium\ (C.r.)$, $Ehrlichia\ chaffeensis\ (E.c.)$, and $Anaplasma\ marginale\ (A.m.)$.

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Figures 2A-2C shows the DNA sequence of the 28 kDa gene locus cloned from *E. chaffeensis* (Fig. 2A-2B) and *E. canis* (Fig. 2C). One letter amino acid codes for the deduced protein sequences are presented below the nucleotide sequence. The proposed sigma-70-like promoter sequences (38) are presented in bold and underlined text as -10 and -35 (consensus -35 and -10 sequences are TTGACA and TATAAT, respectively). Similarly, consensus ribosomal binding sites and transcription terminator sequences (bold letter sequence) are identified. G-rich regions identified in the *E. chaffeensis* sequence are underlined. The conserved sequences from within the coding regions selected for RT-PCR assay are identified with italics and underlined text.

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Figure 3A shows the complete sequence of the MAP2 homolog of *Ehrlichia canis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

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Figure 3B shows the complete sequence of the MAP2 homolog of *Ehrlichia chaffeensis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

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Brief Description of the Sequences

SEQ ID NO. 1 is the coding sequence of the MAP1 gene from *Cowdria ruminantium* (Highway isolate).

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SEQ ID NO. 2 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1.

SEQ ID NO. 3 is the coding sequence of the MAP1 gene from *Ehrlichia chaffeensis*.

SEQ ID NO. 4 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

SEO ID NO. 5 is the *Anaplasma marginale* MSP4 gene coding sequence.

SEQ ID NO. 6 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

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SEQ ID NO. 7 is a partial coding sequence of the VSA1 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 8 is the coding sequence of the VSA2 gene from Ehrlichia chaffeensis. also shown in Figures 2A-2B.

SEQ ID NO. 9 is the coding sequence of the VSA3 gene from *Ehrlichia chaffeensis*. also shown in Figures 2A-2B.

SEQ ID NO. 10 is the coding sequence of the VSA4 gene from Ehrlichia chaffeensis. also shown in Figures 2A-2B.

SEQ ID NO. 11 is a partial coding sequence of the VSA5 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 12 is the coding sequence of the VSA1 gene from *Ehrlichia canis*, also shown in Figure 2C.

SEQ ID NO. 13 is a partial coding sequence of the VSA2 gene from *Ehrlichia canis*. also shown in Figure 2C.

SEQ ID NO. 14 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 7, also shown in Figures 2A-2B.

SEQ ID NO. 15 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 8. also shown in Figures 2A-2B.

SEQ ID NO. 16 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 9. also shown in Figures 2A-2B.

SEQ ID NO. 17 is the polypeptide encoded by the polynucleotide of SEQ ID NO 10. also shown in Figures 2A-2B.

SEQ ID NO. 18 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 11. also shown in Figures 2A-2B.

SEQ ID NO. 19 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 12. also shown in Figure 2C.

SEQ ID NO. 20 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 13. also shown in Figure 2C.

SEQ ID NO. 21 is the coding sequence of the MAP2 gene from *Ehrlichia canis*, also shown in Figure 3A.

SEQ ID NO. 22 is the coding sequence of the MAP2 gene from *Ehrlichia chaffeensis*. also shown in Figure 3B.

SEQ ID NO. 23 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 21. also shown in Figure 3A.

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SEQ ID NO. 24 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 22. also shown in Figure 3B.

SEQ ID NO. 25 is the coding sequence of the map2 gene from Cowdria ruminantium.

SEQ ID NO. 26 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 25.

SEQ ID NO. 27 is the coding sequence of the ihworf3 gene from Cowdria ruminantium.

SEQ ID NO. 28 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 27.

SEQ ID NO. 29 is the coding sequence of the 4hworfl gene from Cowdria ruminantium.

SEQ ID NO. 30 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 29.

SEQ ID NO. 31 is the coding sequence of the 18hworf1 gene from Cowdria ruminantium.

SEQ ID NO. 32 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 31.

SEQ ID NO. 33 is the coding sequence of the 3gdorf3 gene from Cowdria ruminemtium.

SEQ ID NO. 34 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 33.

Detailed Disclosure of the Invention

In one embodiment, the subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response.

According to the subject invention, recombinant DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where an immune response is induced. Prokaryotic signal sequences may be deleted from the nucleic acid encoding an antigen of interest. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal. See, for example, Wolff, J.A., J.J. Ludike, G. Acsadi, P. Williams, A. Jani (1992) *Hum. Mol. Genet.* 1:363. A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

In one embodiment, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been

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shown to be protective against *Cowdria ruminantium*, the causative agent of heartwater in domestic ruminants. Accordingly, nucleotide sequences of rickettsial genes, as described herein, can be used as nucleic acid vaccines against human and animal rickettsial diseases.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine. In another embodiment of the invention, the polynucleotide vaccine is administered in the form of a "cocktail" which contains at least two of the nucleic acid vaccines of the subject invention. The "cocktail" may be administered in conjunction with an antigen or an antigen booster as described above.

The MAP1 gene, which can be used to obtain this protection, is also present in other rickettsiae including *Anaplasma marginale*, *Ehrlichia canis*, and in a causative agent of human ehrlichiosis, *Ehrlichia chaffeensis* (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] *Infect. Immun.* 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain *Rickettsia* spp. MAP1-like genes from *Ehrlichia chaffeensis* and *Ehrlichia canis* have now been cloned and sequenced. These MAP-1 homologs are also referred to herein as Variable Surface Antigen (VSA) genes.

The present invention also concerns polynucleotides encoding MAP2 or MAP2 homologs from *Ehrlichia canis* and *Ehrlichia chaffeensis*. MAP2 polynucleotide sequences of the invention can be used as vaccine compositions and in diagnostic assays. The polynucleotides can also be used to produce the MAP2 polypeptides encoded thereby.

The subject invention further concerns the genes designated Cowdria ruminantium map 2. Cowdria ruminantium 1hworf3. Cowdria ruminantium 4hworf1. Cowdria ruminantium 18hworf1. and Cowdria ruminantium 3gdorf3 and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid vaccine vectors (plasmids), which are commercially available (e.g., Vical, San Diego, CA). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's Remington's Pharmaceutical Science. Mack Publishing Company, Easton, PA.

The subject invention also concerns polypeptides encoded by the subject polynucleotides. Specifically exemplified are the polypeptides encoded by the MAP-1 and VSA genes of *C. rumimontium*, *E. chaffeensis*, *E. canis* and the MP4 gene of *Anaplasma marginale*. Polypeptides uncoded by *E. chaffeensis* and *E. canis* MAP2 genes are also exemplified herein.

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Also encompassed within the scope of the present invention are fragments and variants of the exemplified polynucleotides and polypeptides. Fragments would include, for example, portions of the exemplified sequences wherein procaryotic signal sequences have been removed. Examples of the removal of such sequences are given in Example 3. Variants include polynucleotides and/or polypeptides having base or amino acid additions, deletions and substitutions in the sequence of the subject molecule so long as those variants have substantially the same activity or serologic reactivity as the native molecules. Also included are allelic variants of the subject polynucleotides. The polypeptides of the present invention can be used to raise antibodies that are reactive with the polypeptides disclosed herein. The polypeptides and polynucleotides can also be used as molecular weight markers.

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Another aspect of the subject invention concerns antibodies reactive with MAP-1 and MAP2 polypeptides disclosed herein. Antibodies can be monoclonal or polyclonal and can be produced using standard techniques known in the art. Antibodies of the invention can be used in diagnostic and therapeutic applications.

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In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus(HCMV) enhancer-promoter. In a specific example, this vaccine was injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50 µl/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCL1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and C. ruminantium antigens in in vitro These proliferating cells from mice immunized with lymphocyte proliferation tests. VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100 µg/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of C. ruminantium. Survival rates of 0% to 3% (1/144 survivors/totalin all control groups) were recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, in a specific embodiment, the subject invention

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concerns the discovery that the gene encoding the MAP1 protein induces protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, e.g., whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*. Stockton Press, New York, NY., pp. 169-170.

Examples of various stringency conditions are provided herein. Hybridization of immobilized DNA on Southern blots with 32P-labeled gene-specific probes can be performed by standard methods (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. New York.). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz et al.

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et al. [1983] Methods of Enzymology, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

 $Tm=81.5\,^{\circ}C+16.6\ Log[Na+]+0.41(\%G+C)-0.61(\%formamide)-600/length\ of\ duplex\ in\ base\ pairs.$

Washes are typically carried out as follows:

- (1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash):
- (2) once at Tm-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization can be carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE. 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes can be determined by the following formula:

Tm (°C)=2(number T/A base pairs) +4(number G/C base pairs) (Suggs et al. [1981] ICN-UCLA Symp. Dev. Biol. Using Purified Genes, D.D. Brown [ed.], Academic Press. New York, 23:683-693).

Washes can be carried out as follows:

- (1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash:
- (2) once at the hybridization temperature for 15 minutes in 1X SSPE. 0.1% SDS (moderate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low:

1 or 2X SSPE. room temperature

l or 2X SSPE, 42°C

Moderate:

0.2X or 1X SSPE. 65°C

High:

0.1X SSPE. 65°C.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given

polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.* (1983) *J. Biol. Chem.* 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

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Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia *Cowdria ruminantium*. The vaccine construct tested was the MAP1 gene of *C. ruminantium* inserted into plasmid VCL1010 (Vical. San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 µg VCL1010/MAP1 DNA (V/M in Table 1 below), or 100, 50 µg VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of *C. ruminantium* and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

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Table 1												
	100 μg V/M	75 μg V/M	50 μg V/M	25 μg V/M	100 μg V	50 μg V	Sal.					
Survived	5	7	5	3	0	()	0					
Died	3	1	3	5	8	8	8					

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The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 µg VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection, 30 mice/group were challenged with 30LD50 of *C. ruminantium* and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

			Table 2			
	V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.
Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls (p<0.05).

Again, as summarized in Table 2, the VCL1010/MAP1 DNA vaccine increased the numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to *C. ruminantium* in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

Example 2 – Cloning and sequence analysis of MAP1 homologue genes of *E. chaffeensis* and *E. canis*

Genes homologous to the major surface protein of *C. ruminantium* MAP1 were cloned from *E. chaffeensis* and *E. canis* by using PCR cloning strategies. The cloned segments represent a 4.6 kb genomic locus of *E. chaffeensis* and a 1.6 kb locus of *E. canis*. DNA sequence generated from these clones was assembled and is presented along with the deduced amino acid

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sequence in Figures 2A-2B (SEQ ID NOs. 7-11 and 14-18) and Figure 2C (SEQ ID NOs. 12-13 and 19-20). Significant features of the DNA include five very similar but nonidentical open reading frames (ORFs) for *E. chaffeensis* and two very similar, nonidentical ORFs for the *E. canis* cloned locus. The ORFs for both *Ehrlichia* spp. are separated by noncoding sequences ranging from 264 to 310 base pairs. The noncoding sequences have a higher A+T content (71.6% for *E. chaffeensis* and 76.1% for *E. canis*) than do the coding sequences (63.5% for *E. chaffeensis* and 68.0% for *E. canis*). A G-rich region -200 bases upstream from the initiation codon, sigma-70-like promoter sequences, putative ribosome binding sites (RBS), termination codons, and palindromic sequences near the termination codons are found in each of the *E. chaffeensis* noncoding sequences. The *E. canis* noncoding sequence has the same feature except for the G-rich region (Figure 2C: SEQ ID NOs. 12-13 and 19-20).

Sequence comparisons of the ORFs at the nucleotide and translated amino acid levels revealed a high degree of similarity between them. The similarity spanned the entire coding sequences, except in three regions where notable sequence variations were observed including some deletions/insertions(Variable Regions I, II and III). Despite the similarities no two ORFs are identical. The cloned ORF 2, 3 and 4 of E. chaffeensis have complete coding sequences. The ORF1 is a partial gene having only 143 amino acids at the C-terminus whereas the ORF5 is nearly complete but lacks 5-7 amino acids and a termination codon. The cloned ORF2 of E. canis also is a partial gene lacking a part of the C-terminal sequence. The overall similarity between different ORFs at the amino acid level is 56.0% to 85.4% for E. chaffeensis, whereas for E. canis it is 53.3%. The similarity of E. chaffeensis ORFs to the MAP1 coding sequences reported for C. ruminantium isolates ranged from 55.5% to 66.7%, while for E. canis to C. ruminantium it is 48.5% to 54.2%. Due to their high degree of similarity to MAP1 surface antigen genes of C. ruminantium and since they are nonidentical to each other, the E. chaffeensis and E. canis ORFs are referred to herein as putative Variable Surface Antigen (VSA) genes. The apparent molecular masses of the predicted mature proteins of E. chaffeensis were 28.75 kDa for VSA2, 27.78 for VSA3, and 27.95 for VSA4, while E. canis VSA1 was slightly higher at 29.03 kDa. The first 25 amino acids in each VSA coding sequence were eliminated when calculating the protein size since they markedly resembled the signal sequence of C ruminantium MAP1 and presumably would be absent from the mature protein.

The amino acid sequence derived from the cloned *E. chaffeensis* MAP1-like gene, and alignment with the corresponding genes of *C. ruminantium* and *A. marginale* is shown in Figure 1.

Example 3

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A further aspect of the subject invention are five additional genes which give protection when formatted as DNA vaccines. These genes are *Cowdria ruminantium map 2*, *Cowdria ruminantium 1hworf3*. *Cowdria ruminantium 4hworf1*. *Cowdria ruminantium 18hworf1*, and *Cowdria ruminantium 3gdorf3*. The DNA and translated amino acid sequences of these five genes are shown in SEQ ID NOS. 25-34.

There is published information showing that gene homologs of all five genes are present in other bacteria. For example, a homolog of *map2* is present in *Anaplasma marginale*, a homolog of *Ihworf3* is present in *Brucella abortus*, homologs of *Ihworf1* are present in *Pseudomonas aeruginosa* and *Coxiella burnetii*, and homologs of *I8hworf1* are present in *Coxiella burnetii* and *Rickettsia prowazekii*. This can be revealed by a search of DNA and protein databases with standard search algorithms such as "Blast". Based on the protective ability of these genes against *Cowdria ruminantium* and their presence in other bacterial pathogens, the subject invention further concerns the use of these genes, their gene products, and the genes and gene products of the homologs as vaccines against bacteria. This includes their use as DNA or nucleic acid vaccines or when formulated in vaccines employing other methods of delivery, *e.g.*, recombinant proteins or synthetic peptides in adjuvants, recombinant live vector delivery systems such as vaccinia (or other live viruses) or *Salmonella* (or other live bacteria). These methods of delivery are standard to those familiar with the field. This also includes vaccines against heartwater disease, vaccines against rickettsial diseases in general and vaccines against other bacteria containing homologs of these genes.

Table 3 shows the protective ability of the 5 genes against death from *Cowdria ruminantium* challenge in mice. Genes were inserted into VR1012 according to the manufacturers instructions(Vical, San Diego) and challenge studies were conducted as described in Example 1. N-terminal sequences which putatively encoded prokaryotic signal peptides were deleted because of the potential for their affects on expression and and immune responses in eukaryotic expression systems or challenged animals. The inserts were as follows: map2. SEQ ID NO. 25. beginning at base 46: 18hworf1. SEQ ID NO. 31, beginning at base 67: 3gdorf3. SEQ ID NO. 33, beginning at base 79: lhworf3, SEQ ID NO. 27, beginning at base 76: and 4hworf1, SEQ ID NO. 29, beginning at base 58.

Table 3													
DNA Construct	MWT	Survival Rate											
	Size	Vacci	nated	Con	P value								
TMMAP 2	21 kd	9/28*	32%	0/29	00.0	0.004							
MB18HWORF1	28 kd	10/30*	33%	1/27	46%	0.021							
AM3GDORF3	16 kd	7/26	27° o	1/27	40.0	0.060							
TM1HWORF3 36 kd		8/29	28%	2/30	7°%	0.093							
TM4HWORF1 19 kd		10/30*	33%	2/30	70.0	0.054							

Control - VR1012 DNA vector plasmid only

*Statistically significant difference (Fisher's Exact test)

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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<u>Claims</u>

1	1. A composition comprising a polynucleotide which encodes a polypeptide having the
2	characteristic of eliciting an immune response protective against disease or death caused by a
3	rickettsial pathogen.
1	2. The composition, according to claim 1, wherein said rickettsial pathogen is selected
2	from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and Cowdria spp.
1	3. The composition, according to claim 1, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6.
3	SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ
4	ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30. SEQ ID NO. 32. SEQ ID NO. 34. homologs
5	thereof. and immunogenic fragments thereof.
1	4. The composition, according to claim 1, wherein said polynucleotide has a nucleic
2	acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.
3	5. SEQ ID NO. 7. SEQ ID NO. 8. SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, . SEQ
4	ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs
5	thereof, and fragments thereof which encode immunogenic polypeptides.
l	5. The composition, according to claim 4, wherein said polynucleotide has a nucleic
2	acid sequence of SEQ ID NO. 3, or a fragment thereof.
1	6. The composition, according to claim 1, wherein said polynucleotide further
2	comprises a nucleic acid vaccine vector.
1	7. The composition, according to claim 1, further comprising a pharmaceutically
2	acceptable carrier.
1	8. A polynucleotide encoding a polypeptide having an amino acid sequence selected
2	from the group consisting of SEQ ID NO. 4, SEQ ID NOS. 14-20, SEQ ID NOS. 23-24, SEQ
3	ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and fragments
4	thereof.

	9. The polynucleotide, according to claim 8, said polynucleotide having a nucleic acid
2	sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NOS. 7-13, SEQ ID
3	NOS. 21-22. SEQ ID NOS. 25. SEQ ID NO. 27. SEQ ID NO. 29. SEQ ID NO. 31. and SEQ ID
1	NO. 33.
l	10. A method for protecting a susceptible host against disease or death caused by a
2	rickettsial pathogen, said method comprising administering an effective amount of a
3	polynucleotide encoding polypeptide having the characteristic of eliciting an immune response
4	protective against said rickettsial pathogen.
1	11. The method, according to claim 10, wherein said rickettsial pathogen is selected
2	from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and Cowdria spp
1	12. The method, according to claim 10, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2. SEQ ID NO. 4, SEQ ID NO. 6
3	SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ
4	ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, or homological
5	thereof and immunogenic fragments thereof.
1	13. The method, according to claim 10, wherein said polynucleotide has a nucleic acid
2	sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5
3	SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID
4	NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, and SEQ ID NO. 33.
1	14. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
2	sequence of SEQ ID NO. 1.
1	15. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
2	sequence of SEQ ID NO. 3.
1	16. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
2	sequence of SEQ ID NO. 5.

1	17. The method, according to claim 10, wherein said nucleic acid further comprises an
2	appropriate nucleic acid vector.
1	18. The method, according to claim 10, wherein said composition further comprises a
2	pharmaceutically acceptable carrier.
1	19. The method, according to claim 10, which further comprises administration to said
2	host of said polypeptide encoded by said polypeptide.
1	20. A method for detecting, in a human or animal, antibodies associated with infection
2	by Ehrlichia, wherein said method comprises contacting a biological fluid from said human or
3	animal with a polypeptide selected from the group consisting of SEQ ID NO. 4, SEQ ID NOS.
4	14-20. SEQ ID NOS. 23-24, SEQ ID NO. 26. SEQ ID NO. 28, SEQ ID NO. 30. SEQ ID NO.
5	32. SEQ ID NO. 34, and homologs and fragments thereof.
1	21. A method of detecting the presence of rickettsial nucleic acids comprising
2	contacting a sample suspected of containing rickettsial nucleic acids with a composition
3	comprising a labeled polynucleotide which encodes a polypeptide having the characteristic of
4	eliciting an immune response protective against disease or death caused by a rickettsial
5	pathogen, allowing for the formation of a hybridization complex and detecting said label.
1	22. The composition, according to claim 21, wherein said rickettsial pathogen is
2	selected from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and
3	Cowdria spp.
1	23. The composition, according to claim 21, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6.
3	SEQ ID NO. 14. SEQ ID NO. 15. SEQ ID NOS. 16-20. SEQ ID NO. 23. SEQ ID NO. 24. SEQ
4	ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30. SEQ ID NO. 32. SEQ ID NO. 34, and homologs
5	and immunogenic fragments thereof.
1	24. The composition, according to claim 21, wherein said polynucleotide has a nucleic
2	acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.
3	5. SEQ ID NO. 7. SEQ ID NO. 8. SEQ ID NOS. 9-13. SEQ ID NO. 21. SEQ ID NO. 22 SEQ

- 4 ID NO. 25. SEQ ID NO. 27. SEQ ID NO. 29. SEQ ID NO. 31. SEQ ID NO. 33. homologs
- 5 thereof, and fragments thereof which encode immunogenic polypeptides.

FIG. 1A

뒤 6. 1B

FIG. 1C

	C.r. A.m. C.r. A.m. C.r. A.m. A.m.	GTGGACATTTCCATAGAGTAATAGGAATTTAAAGATATTGCTACCTTAAAAATAT GTGGGCACTTTCATAAGGTAATAGGAATTTTAGAGATATTCCTACTATAATACCTA GTGGGCACTTTCATAAGGTAATTGATGATGATTTTAGAGACATTCCTACTATAATACCTA ***** TAGGGTTCTACCACGGCTATTTGATGATGATCTTACAAGGACATTCCCGCACACAGGG **** TTACTTCAAAAAAAAAAGGAAAAGGAAACTACCTGCATATTGTC CTGGATCAAAAAAAGGAAAAAACCTAGCAATAGTAATACTGGATGTATGCC TAAAGTTCTCGAGAAAAAAAAAA
	C.r. E.c. A.m.	ACTTTGGTATAGAAATTGGAGGAGGTTTGTATTTTAA ACTACGGCTTTAACCTTGGAGGAGGTTTAA

```
1 ggaatqaattcagggacatttttacttttaaaqcqtttqctacaccatcttqcagcta
     NEFRDISTLKAFATPSSAAT
 51 ctccagacttagcaacagtaacactgagtgtgttccacttttggagtagaacttggaggaa p D L A T V T L S V C H F G V E L G G R
 121 ganttaacttotaatttattattattgoododtgttaadaataatotaadcttgttittatt
     F 3 F *
 -35
361 actaaaaactagcttataacttgtttttacattgtaggtttactactgttaatttgtttt
                -10
421 cactatttc<u>aggtgts</u>acatgaactgcgaaaaattttttacaacaactgcattaacatta
RBS M N C E K F F I T T A L T L
481 ctaatgtccttcttacctggaatatcactttctgatccagtacaggatgacaacattagt
        SPLPGISLSDPVQDDNIS
541 ggtaatttctacatcagtggaaagtatatgccaagcgcttcgcattttggagttttttct
   GNFYISGKYHPSASHPG
601 gccaaggaagaaagaaacacaacagttggagtatttggaacagagcaagattgggataga
    AKEERNTTVGVFGIEQDWDR
661 cgcgtaatatotagaaccactttaagogatatattcaccgttccaaa<u>ttattcatt</u>
    CVISRTTLSDIFTVPNYSFK
721 <u>caccaa</u>aacaatotattttoaggatttgcaggagetattggctactcgattggctca
    YENNLFSGFAGAIGYS HDGP
781 agaatagagottgaagtatotTatgaagcattsgatgttaaaaatcaaggtaacaattat
    RIELEVSYEAPDVKNQGNNY
841 aagaacgaagcacatagatattatgototgtoocatottotoggcacagagacacagata
   KNEAHRYYALSHLLGTETQI
901 gatggtgcaggcagtgcgtctttctaataaatgaaggactacttqataaatcattt
   DGAGSASVFLINEGLLDKSF
MLNACYDVISEGIPFSPYIC
1021 gcaggtattggtattgatttagtatccatgetscaaggtatgaacgstaaaatttottat A G I G I D L V S M F E A I N P K I S Y
1081 caaggaaaattaggettaagttaceetataageeetagaagettetgtgtttattggtgga Q G K L G L S Y P I S P E A S V F I G G
1141 cattttcataaggtgataggaaacgaatttagaçatattcctactatgatacctagtgaa
   H F H K V I G N E F R D I P T M I P S E
1201 toagogottgcaggaaaaggaaactaccctgcaatagtaacactggacgtgttctacttt
       LAGKGNYPAIVTLDVFYF
GIELGGRENPQL
1321 atagtggcmaagaatgtagcaataagaggggggaggggaactaaattattatttgcc
1381 atatecettaetaecaettaeaecaaataatetgaeaaataeaaeagtteaaacaaggt
1441 aaacaattottaaatttgtottatgagaaccsttgttatattatataaaaactagotta
                               -35
1501 taac ttgtctttacattgcacttctactattgttaatttattttcactattttaqqtqca
    -10
1561 atatgaattgcaaaaaatttttttataacaactgcattagtatcactaatgtcctttctac
     MNCKKFFITTALVSLMSFLP
1621 orggaatateattttotgatooagtgcaaqgtgacaatattagtggtaatttotatgtta
     G I S F S D P V Q G D N I S G N F Y V S
1681 gtggcaagtatatgccaagtgcttcgcatttttggcatgttttctgccaaagaagaaaaaa
     G KYMPSASH F G M P S A K E E K N
1741 atcotactgctgcattgtatggcttaaaacaacattgggaagggattagctcatcaagtc P T V \lambda L Y G L \kappa Q D \omega E G I S S S S H
1801 acaacgataatcatttcaattacaaggg<u>ttastsasttsaatatcaa</u>aataacccatttt
N D N H P N N K G Y S F K Y E N N P F L
                                  <u>caaacaccaa</u>aataacccatttt
1921 cotatgaaacatttgacgttaaaaatcagggtaataactataaaaatgatgctcacagat
     YETFOVKNQGNNYKNDAHRY
1981 actgcgctttaggccaacaagacaacagcgcaatacctaaaactagtaaatacgtactgt
     CALGQQDNSGIPKTSKIVLL
2041 taaaaaggaaggattgettgacatateatttatgetaaatgcatgctatgatataataa K S E G L L D I S F M L N A C Y D I I N
2101 acgagagcatacctttgtctccttacatatgtgcaggtgttggtActgatttaatatcca
                                  VGTDLIS
     ESIPLSPYICAG
2161 tettecaagetagaateetaaaattitttactaaggaagttaggtotaagttactata F E A T N P K I S Y Q G K L G L S Y S I
2221 taaacccagaagcttctgtatttattggtggacattttcataaggtgataggaaacgaat
     NPEASVFIGGHFHKV
                                         IGNEF
2281 ttagggacattoctactctgaaagcatttgttacqtcatcagctactccagatctagcaa
     RDIPTLKAFVTSSÄTPDLÄI
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FIG. 2A

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5/8 2401 tttgttattgccacatgttaaaaataacctaaacttgttttcattattgctacagtaaac 2521 accatatocottattatacoacttacactaaacaacttgacaaacacaacagcttctgga 2581 aaaacaaacaatacttaaatttctctttacaaaaaccatttataccttgtactaaaaacta -35 2641 gettataacttpttttttacattgtagttetactattgttaatttattttcactatttta 2701 gtgcsatatgaattgcsaaaattttttataacaactacattagtatcgctsatgtcctt HNCKKFFITTLVSLMSP RBS 2821 tatcagtgggaaatatgtaccaagtgttttaacatttttggcgtattctctgctaaacagga I S G K Y V P S V S H F G V F S A K Q E 2881 aagaaacacaaccggagtatttggattaaagcaagattgggatggcagcacaatatcR N T T I G V F G L K Q D W D G S T I S 2941 taaaaattotoosgaaaatacatttaacgttoosaactactoscosaaacaccaaaataa KNSPENTFNVPN7SFKYENN 3001 tocatttetaggttttgcaggagetgtttggttatttaatgaatggtccaagaatagagtt P F L G P A G A V G Y L M N G P R I E L 3061 agaaatgteetatgaaacatttgatgtgaaaaactagggtaataactataagaacgatge E M S Y E T F D V K N Q G N N Y K N D A 3121 teacaaatattatgetttaacceataacagtgggggaaagetaagcaatgcaggtgataa H K, Y Y A L T H N S G G K L S N A G D K 3181 gettgtttttetamamatgamggmetmettgmtatatemettatgttgmmtgemtgeta FVFLKNEGLLDISLMLNAC 3241 tgatgtaataagtgaaggaatacctttctctctcttcttacatatgtgcaggtgttggtactga
D V I S E G I P F S P Y I C A G V G T D 3301 tttaatateeat $\underline{\sigma}$ cttaaa $\underline{\sigma}$ cttaaa $\underline{\sigma}$ cttaaaa $\underline{\sigma}$ cttaaaaatttettateaaggaaagttaggttt L I S M F E A I N P K I S Y Q G K L G L 3421 agggaatgaattcagagatattcctgctatgatacccagtacctcaactctcacaggtaa GNEPRDIPAMIPSTSTLTGN 3481 toactitaciatagtaacactaagtgtatgccactttggagtggaacttggaggaaggtt H F T I V T L S V C H F G V E L G G R F 3541 taacttttaattttattattgccacatgttaaaaattaatccaaacttgttttattattg N P: * 3721 tattacttacctgacgtaatatattaaattttccttacaaaagttaccgatattttatac -35 3781 aaaaatt<u>tattetgacttgctttatatgacacttcta</u>ctattgctaatttacttgcc -10 3841 actattaggttatatatgaattacaaaaagttttcataacaagtgcattgatatcatta MNYKKVFITSALISL P.BS 3901 atatottototacotggagtatoattttoogacopagcaggtagtggtattaacggtaat ISSLPGVSFSDPAGSGINGN 3961 ttotacateagtggaaaatacatgccaagtgcttogcattttggagtattctctgccaag FY I S G K Y M P S A S H F G V F S A K 4021 gaagaaagaaatacaacagttggagtgttttggactgaagcaaaattgggacggaagcgca E E R N T T V G V F G L K Q N W D G S λ 4081 atatecaacteeteeccaaacgatgtatteactgteteaaa<u>ctateessteaaacaccaa</u> ISNSSPNDVFTVSNYSF 4141 aacaaccegittttaggitttgcaggagctattggttactcaatggatggtccaagaata N N P F L G F A G A I G Y S M D G P R I 4201 gagettgaagtatettatgaaacatttgatgtaaaaaatcaaggtaacaattataagaat ELEVSYETFDVKNQGNNYKN 4261 gaagcacatagatattgtgctctatcccataactcagcagcagacatgagtagtgcaagt EAHRYCALSHNSAADMSSAS 4321 aataattttgtotttotaaaaaatgaaggattaottgacatateatttatgotgaacgca N N F V F L K N E G L L D I S F M L N A 4331 tgctatgacgtagtaggcgaaggcatacctttttttccttatatatgcgcaggtatcggt C Y D V V G E G I P F S P Y I C A G I G 4441 actgatttagtatccat<u>scttsaacctacaaacc</u>ttaaaaatttettactaaggaaagtta
T D L V S M F E A T N P K I S Y Q C K L
4501 ggtttaagctactataaagcccagaagcttctgtggtggcactttataag
G L S Y S I S P E A S V F I G G H F H K 4561 gtaatagggaacgaatttagagatattectactataatacctactggatcaacacttgca V I G N E F R D I P T I I P T G S T L A 4621 ggaaaaggaaactaccctgcaatagtaatactggacgtatgccactttggaatagaaatg G K G N Y P A I V I L D V C H F G I E M 4681 gga

FIG. 2B

```
51 ctttacacattttatacctttttatagtccagcacgtgccagtacaattcacaacttcta
F T H F I P F Y 3 P A R A S T I H N F Y
 121 cattagtggaaaatatatqccaacagcgtcacattttggaattttttttggctaaaqaaga
 181 acaaagttttactaaggtattagttgggttagatcaacgattatcacataatattataaa
            TKVLVGLDQRLSHNII
     0 5 ?
 241 caataatgatacagcaaagagtottaaggttttaaattatttatttaaatacaaaaataa
     NNDTAKSLKVQNYSFKYKNN
 301 cocatttctaggatttqcagqaqctattggttattcaataggcxxttcaaqaataqaact
     PFLGFAGAIG
                             YSIG
 361 agaagtatcacatgaaatatttgatactaaaaacccaggaaacaactatttaaatgactc
     EVSHEIFDIKNPGNNYLNDS
 421 toacamatattgcgctttatctcatggmagtcacatatgcmgtgatggmaatagcggmgm
     H K Y C A L S H G S H I C S D G N S
 481 ttggtacactgcaaaaactgataagtttgtacttctgaaaaatgaaggtttacttgacgt W Y T A K T D K P V L L K N E G L L D V
 541 ctcatttatgttaaacgcatgttatgacataacaactgaaaaaatgcctttttcacctta
     S F M L N A C Y D I T T E K M P F S P Y
 601 tatatgigdaggtatiggtadigatotdatatotzigitigagadaacacaaaacaaaat
     ICAGIGTDLISMFETTQNKI
 661 atottatcaaggaaagttaggtttaaactatactataaactcaagagtttctgtttttgc
     SYQGKLGLNYTINSRVSVFA
 721 aggtgggcactttcataaggtaataggtaatgaatttaaaggtattcctactotattacc
     GGHFHKVIGNEFKGIPTLLP
 781 tgatggatcaaacattaaagtacaacagtctgcaacagtaacattagatgtgtgccattt
     DGSNIKVQQSATVTLDVCHP
 841 cgggttagagattggaagtagattttttttttaatacttctattgtacatgttaaaaata
     G L E I G S R F F F +
 901 gtadtagtttgdttdtgtggtttataaacgcaagagagaaatagttagtaataaattaga
 1021 tataaatgttacttattaataattttacgtagtatattaaattttcccttacaaaagccac
1081 tagtattttatactaaaagctatactttggcttgtatttaatttttactactgt
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                      -10
1141 taatttactttcactgtttc<u>tqvtq</u>taaatatçaattgtaaaaaagttttcacaataagt
                           HNCKKVFTIS
1201 gcattgatatcatccatatacttcctacctaatgtctcatactctaacccagtatatggt
    ALISSIYFLPNVSYSNPVYG
1261 aacagtatgtatggtaatttttacatatcaggaaagtacatgccaagtgttoctcatttt
    NSMYGNFYISGKYMPSVPHF
1321 ggaattttttcagctgaagaagaagaaaaaaaagacaactgtagtatatggcttaaaagaa G I F S A E E E K K K T T V V Y G L K E
1381 aactgggcaggagatgcaatatctagtcaaagtccagatgataattttaccattcgaaat
    N W A G D A I S S Q S P D D N F T I R N
1441 tactoattcaagtatgcaagcaacaagtttttagggtttgcagtagctattggttactcg
    YSFKYASNKFLÖFAVÄIĞYS
1501 ataggcagtccaagaatagaagttgagatgtcttatgaagcatttgatgtaaaaaatcaa I G S P R I E V E M S Y E A P D V K N Q
1561 ggtaacaatt
    GNN
```

FIG. 2C

+	acacycacactactactactactactactactcactaagttaaatt
61	ataccattctcttttcactttatcagaagacttttattta
121	tgtcacaaataaacacactgcaactgcaatcactacgtaaaactttaactcttctttt
181	acaactaaaatactaataaaagtaatatagtataaaaaatottaagtaac <u>TTGACA</u> taa -35
241	attactctgata <u>TAGCAT</u> atgtctagtatctctatactaaacgtttatatatatt <u>GGAG</u> c;
301	tattaATGAAAGCTATCAAATTCATACTTAATGTCTGCTTACTATTTGCAGCAATATTTT M K A I K F I L N V C L L F A→ A I F
361	TAGGGTATTCCTATATTACAAAACAAGGCATATTTCAAACAAA
	G Y S Y I T K Q G I F Q T K H H D T P M
421	ATACTACTATACCAAATGAAGACGGTATTCAATCTAGCTTTAGCTTAATCAATC
	TTIPNEDGIQSSFSLINQDO
481	GTAAAACAGTAACCAGCCAAGATTTCCTAGGGAAACACATGTTAGTTTTGTTTTGGATTCT
541	CTGCATGTAAAAGCATTTGCCCTGCAGAATTGGGATTAGTATCTGAAGCACTTGCACAAG
	ACKSICPAELGLVSEALAQI
601	TTGGTAATAATGCAGACAAATTACAAGTAATTTTATTACAATTGATCCAAAAAATGATAG N N A D K L Q V I F I T I D P K N D T
661	CTGTAGAAAATTAAAAGAATTTCATGAACATTTTGATTCAAGAATTCAAATGTTAACAG
	V E K L K E F H E H F D S R I Q M L T G
721	GAAATACTGAAGACATTAATCAAATAATTAAAAATTATAAAATATATGTTGGACAAGCAG N T E D I N Q I I K N Y K I Y V G Q A D
701	
781	ATAAAGATCATCAAATTAACCATTCTGCAATAATGTACCTTATTGACAAAAAAGGATCAT K D H Q I N H S A I M Y L I D K K G S Y
841	ATCTTTCACACTTCATTCCAGATTTAAAATCACAAGAAAATCAAGTAGATAAGTTACTAT
	LSHFIPDLKSQENQVDKLLS
901	CTTTAGTTAAGCAGTATCTGTAAtttaataattaatt <u>AAAG</u> agaatagtacaca <u>CTTT</u> tt L V K Q Y L *
961 1021	ataaattcatggaatacgttggatgzgtzggttzzttttagtatttttagtgctaataac

FIG. 3A

				_		-				-										
61	caaa	aaa	aac	tt	tac	aac	tta	atta	tgt	itti	atet	ctaa	aaa	cct	tat	ttta	aaga	atto	ett	atç
121	tcad	aa	aat	aac	:aaa	aat	act	att	tac	caaa	aata	acad	cad	caa	ttt	cato	caaa	ataa	ıaaa	ı a a a
181	ctat	ac	act	tta	tta	tac	tac	agt	aga	itat	acc	ata	aaa	aga	ttt	taaq	gtaa		<u>GAC</u>	<u>A</u> ta
241	atat	ta	cct	tgg	rta]	<u>'AGC</u> 1-		tga	ttt	agt	att	:tta	itat	ta	aaa	ttta	atta			GGA
301	<u>G</u> cat	aa						lati F										AGC →A		
361	TCTA L	AGG! G		TTC														AAT) N		
421	CAAT N	TAC	aaa N	TAT I	ATC S			AGC A										AAA N		AGA D
481	TGG? G																	ATT F		
541	TTCT S																	TCT L		ACA Q
601	GCTT L							GTT L								TGA D			AAA N	
661	TACT T								-	-								AAT M		
721	AGGC G			AGA E				AAA K										TGG G		
781	AGAI D																	AAA K		
841	ATAC Y	AT:						AGA D										TAA K		
901	ATCT S							CTA	Att	taa	taa	tta	att	a <u>A</u> A	<u>GAG</u>	aat	agt	aca	са <u>С</u>	<u>rct</u>
961 021	<u>T</u> ata		aat	tca	tgg	ata	tat	gtg	atş	ggt	аţа	tt	ctt	ttg	gtg	ttt	cta	teş	cta	att

FIG. 3B

I

SEQUENCE LISTING

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Ser	Phe cca		cct Pro 20	ggt Gly agt	gtg Val gtt	tcc Ser	ttt Phe att	tct Ser 25	Ser 10 gat Asp	Thr gta Val aaa	ata Ile tac	Ile cag Gln atg	gaa Glu 30 cca	Leu 15 gac Asp	agc Ser gca	96 144
ser aac Asn	Phe cca Pro	gca Ala 35 ttt	cct Pro 20 ggc Gly	5 ggt Gly agt Ser	gtg Val gtt Val	tcc Ser tac Tyr	ttt Phe att Ile 40	tct Ser 25 agc Ser	Ser 10 gat Asp gca Ala	Thr gta Val aaa Lys	ata Ile tac Tyr	cag Gln atg Met 45	gaa Glu 30 cca Pro	Leu 15 gac Asp act Thr	agc Ser gca Ala	
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Asp	Ser	Ser	Asn	Thr 85	Asn	Ser	Thr	Ile	Phe 90	Thr	Glu	Lys	Asp	Tyr 95	Ser	
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tac Tyr	tca Ser	atg Met 115	aat Asn	gga Gly	cca Pro	aga Arg	ata Ile 120	gag Glu	ttc Phe	gaa Glu	gta Val	tcc Ser 125	tat Tyr	gaa Glu	act Thr	384
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act Thr	tca Ser	aaa Lys	aca Thr 260	Gly	ata Ile	tct Ser	aat Asn	cct Pro 265	ggc Gly	ttt Phe	gca Ala	tca Ser	gca Ala 270	aca Thr	ctt Leu	816
gat Asp	gtt Val	tgt Cys 275	His	ttt Phe	ggt Gly	ata Ile	gaa Glu 280	att Ile	gga Gly	gga Gly	agg Arg	ttt Phe 285	gta Val	ttt Phe	taa	864

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275

280

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WO 00/65063 PCT/US00/10886

5

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Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp 100 105 110

Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys

Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu 130 135 140

Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val 145 150 155 160

Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala 165 170 175

Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys 180 185 190

Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro 195 200 205

Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro 210 215 220

Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn 225 230 235 240

Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala 245 250 255

Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe 260 265 270

Gly Ile Glu Met Gly Gly Arg Phe 275 280

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_	_			_			acc Thr			-	_			_	_	240
_		-	_		-		agt Ser	-								288
				_			aaa Lys									336
		-					gga Gly 120									384
							gcg Ala									432
	-		Leu	Ala	_	Ile	acc Thr	Arg	Asp	Ala				-		480
			_	_			gat Asp	_						_		528
				165					170					175		
				tat			ctg Leu		aca					tcc		576
Leu tat	Asn gta	Gly tgt	Cys 180 gcc	tat Tyr ggg	Asp ata	Val ggc		His 185 agc	aca Thr	Asp gtt	Leu gac	Pro atc	Val 190 tct	tcc Ser	Pro caa	576 624

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Ser His	Glu 35		Ala	Ser	Glu	Gly 40	Gly	Val	Met	Gly	Gly 45	Ser	Phe	Tyr	
Val Gly 50		Ala	Tyr	Ser	Pro 55	Ala	Phe	Pro	Ser	Val 60	Thr	Ser	Phe	Asp	
Met Arg 65	Glu	Ser	Ser	Lys 70	Glu	Thr	Ser	Tyr	Val 75	Arg	Gly	Tyr	Asp	Lys 80	
Ser Ile	e Alā	Thr	Ile 85		Val	Ser	Val	Pro 90	Ala	Asn	Phe	Ser	Lys 95	Ser	
Gly Tyr	Thr	Phe		Phe	Ser	Lys	Asn 105		Ile	Thr	Ser	Phe	Asp	Gly	
Ala Val	Gly		Ser	Leu	Gly	Gly 120		Arg	Val	Glu	Leu 125	Glu	Ala	Ser	
Tyr Arg		, Phe	Ala	Thr	Leu 135		Asp	Gly	Gln	Tyr 140	Ala	Lys	Ser	Gly	
Ala Gli 145	ı Ser	Leu	ı Ala	Ala 150		Thr	Arg	Asp	Ala 155		Ile	Thr	Glu	Thr 160	
Asn Ty	r Phe	e Val	. Val		: Ile	Asp	Glu	11e	Thr	Asn	Thr	Ser	Val 175	Met	

Leu Asn Gly Cys Tyr Asp Val Leu His Thr Asp Leu Pro Val Ser Pro 180 185 190

Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Val Asp Ile Ser Lys Gln 195 200 205

Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Val Gly Ile Ser Tyr Gln 210 215 220

Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gly Phe Tyr His Gly Leu 225 230 235 240

Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala His Asn Ser Val Lys Phe 245 250 255

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<213> Ehrlichia chaffeensis

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<210> 11

<211> 830

<212> DNA

<213> Ehrlichia chaffeensis

<400> 11

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ttctaaaaaa tgaaggatta cttgacatat catttatget gaacgcatge tatgacgtag 540 taggegaagg catacettt teteettata tatgegeagg tateggtaet gatttagtat 600 ceatgtttga agetacaaat cetaaaattt ettaceaagg aaagttaggt ttaagetaet 660 ctataageec agaagettet gtgtttattg gtgggeaett teataaggta atagggaaeg 720 aatttagaga tatteetaet ataataeeta etggateaae aettgeagga aaaggaaaet 780 aecetgeaat agtaataetg gatgtatgee aetttggaat agaaatggga 830

<210> 12

<211> 864

<212> DNA

<213> Ehrlichia canis

<400> 12

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<210> 13

<211> 399

<212> DNA

<213> Ehrlichia canis

<400> 13
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caggaaagta catgccaagt gttcctcatt ttggaatttt ttcagctgaa gaagagaaaa 180
aaaagacaac tgtagtatat ggcttaaaag aaaactgggc aggagatgca atatctagtc 240
aaagtccaga tgataatttt accattcgaa attactcatt caagtatgca agcaacaagt 300
ttttagggtt tgcagtagct attggttact cgataggcag tccaagaata gaagttgaga 360
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<210> 14

<211> 43

<212> PRT

<213> Ehrlichia chaffeensis

<400> 14

Asn Glu Phe Arg Asp Ile Ser Thr Leu Lys Ala Phe Ala Thr Pro Ser

1 10 15

Ser Ala Ala Thr Pro Asp Leu Ala Thr Val Thr Leu Ser Val Cys His
20 25 30

Phe Gly Val Glu Leu Gly Gly Arg Phe Asn Phe 35

<210> 15

<211> 286

<212> PRT

<213> Ehrlichia chaffeensis

<400> 15

Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu Met 1 5 10 15

Ser Phe Leu Pro Gly Ile Ser Leu Ser Asp Pro Val Gln Asp Asp Asn 20 25 30

Ile Ser Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser 35 40 45

His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly 50 55 60

Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys Val Ile Ser Arg Thr
65 70 75 80

Thr Leu Ser Asp Ile Phe Thr Val Pro Asn Tyr Ser Phe Lys Tyr Glu 85 90 95

Asn Asn Leu Phe Ser Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp 105 100

Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Ala Phe Asp Val Lys 120

Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Tyr Ala Leu 135

Ser His Leu Leu Gly Thr Glu Thr Gln Ile Asp Gly Ala Gly Ser Ala 155

Ser Val Phe Leu Ile Asn Glu Gly Leu Leu Asp Lys Ser Phe Met Leu 165 170

Asn Ala Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr 185

Ile Cys Ala Gly Ile Gly Ile Asp Leu Val Ser Met Phe Glu Ala Ile 200

Asn Pro Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Pro Ile 215

Ser Pro Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile 235 230

Gly Asn Glu Phe Arg Asp Ile Pro Thr Met Ile Pro Ser Glu Ser Ala 245 250

Leu Ala Gly Lys Gly Asn Tyr Pro Ala Ile Val Thr Leu Asp Val Phe 265 260

Tyr Phe Gly Ile Glu Leu Gly Gly Arg Phe Asn Phe Gln Leu 280

<210> 16

<211> 278

<212> PRT

<213> Ehrlichia chaffeensis

Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu Met 10

Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Pro Val Gln Gly Asp Asn 25

Ile Ser Gly Asn Phe Tyr Val Ser Gly Lys Tyr Met Pro Ser Ala Ser 3.5 40

His Phe Gly Met Phe Ser Ala Lys Glu Glu Lys Asn Pro Thr Val Ala 55

Leu 65	Tyr	Gly	Leu	Lys	70	Asp	Trp	Glu	GIY	75	Ser	ser	ser	ser	80
Asn	Asp	Asn	His	Phe 85	Asn	Asn	Lys	Gly	Tyr 90	Ser	Phe	Lys	Tyr	Glu 95	Asn
Asn	Prc	Phe	Leu 100	Gly	Phe	Ala	Gly	Ala 105	Ile	Gly	Tyr	Ser	Met 110	Gly	Gly
Pro	Arg	Val 115	Glu	Phe	Glu	Val	Ser 120	Tyr	Glu	Thr	Phe	Asp 125	Val	Lys	Asn
Gln	Gly 130	Asn	Asn	Tyr	Lys	Asn 135	Asp	Ala	His	Arg	Tyr 140	Cys	Ala	Leu	Gly
145			Asn		150					155					160
_			Gly	165					170					175	
			Asn 180					185					190		
		195	Asp				200					205			
	210		Gly			215					220				
225			Ile		230					235					240
			Pro	245					250					255	
			Ile 260			Leu	Ser	Val 265	Cys	His	Phe	Gly	11e 270	Glu	Leu
Gly	Gly	Arg 275	Phe	Asn	Phe										
<21	0 > 1	7													
	1 > 2 2 > P														
			chia	cha	ffee	nsis									
	0> 1 Asn		Lys	Lys 5	Phe	Phe	Ile	Thr	Thr	Thr	Leu	Val	Ser	Leu 15	Met
Ser	Ph∈	Leu	Pro 20	Gly	Ile	Ser	Phe	Ser 25	Asp	Ala	Val	Gln	Asn 30	Asp	Asn

WO 00/65063 PCT/US00/10886

Val Gly Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Val Pro Ser Val Ser

His Phe Gly Val Phe Ser Ala Lys Gln Glu Arg Asn Thr Thr Ile Gly

Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser Thr Ile Ser Lys Asn 65 70 75 80

Ser Pro Glu Asn Thr Phe Asn Val Pro Asn Tyr Ser Phe Lys Tyr Glu 85 90 95

Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Val Gly Tyr Leu Met Asn

Gly Pro Arg Ile Glu Leu Glu Met Ser Tyr Glu Thr Phe Asp Val Lys
115 120 125

Asn Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Lys Tyr Tyr Ala Leu

Thr His Asn Ser Gly Gly Lys Leu Ser Asn Ala Gly Asp Lys Phe Val 145 150 155 160

Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Leu Met Leu Asn Ala 165 170 175

Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys 180 185 190

Ala Gly Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Ile Asn Pro 195 200 205

Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro 210 215 220

Glu Ala Ser Val Phe Val Gly Gly His Phe His Lys Val Ile Gly Asn 225 230 235 240

Glu Phe Arg Asp Ile Pro Ala Met Ile Pro Ser Thr Ser Thr Leu Thr

Gly Asn His Phe Thr Ile Val Thr Leu Ser Val Cys His Phe Gly Val 260 265 270

Glu Leu Gly Gly Arg Phe Asn Phe

<210> 18

<211> 276

<212> PRT

<213> Ehrlichia chaffeensis

<400> 18

Met	Asn	Tyr	Lys	Lys	Val	Phe	Ile	Thr	Ser	Ala	Leu	Ile	Ser	Leu	Ile
1				5					10					15	

- Ser Ser Leu Pro Gly Val Ser Phe Ser Asp Pro Ala Gly Ser Gly Ile 20 25 30
- Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser His
 35 40 45
- Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly Val
 50 55 60
- Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser Ser 65 70 75 80
- Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu Asn 85 90 95
- Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp Gly
 100 105 110
- Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn 115 120 125
- Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu Ser 130 135 140
- His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val Phe 145 150 155 160
- Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys
 165 170 175
- Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys Ala 180 185 190
- Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro Lys 195 200 205
- Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro Glu 210 215 220
- Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu 225 230 235 240
- Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala Gly 245 250 255
- Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe Gly 260 265 270

Ile Glu Met Gly 275

<210> 19 <211> 287 <212> PRT <213> Ehrlichia canis <400> 19 Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu Val Leu Leu Thr Ser Phe Thr His Phe Ile Pro Phe Tyr Ser Pro Ala Arg Ala Ser Thr 2.5 Ile His Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Thr Ala Ser His Phe Gly Ile Phe Ser Ala Lys Glu Glu Gln Ser Phe Thr Lys Val Leu Val Gly Leu Asp Gln Arg Leu Ser His Asn Ile Ile Asn Asn Asn Asp 75 Thr Ala Lys Ser Leu Lys Val Gln Asn Tyr Ser Phe Lys Tyr Lys Asn 90 85 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Ile Gly Asn Ser Arg Ile Glu Leu Glu Val Ser His Glu Ile Phe Asp Thr Lys Asn 115 120 Pro Gly Asn Asn Tyr Leu Asn Asp Ser His Lys Tyr Cys Ala Leu Ser 130 His Gly Ser His Ile Cys Ser Asp Gly Asn Ser Gly Asp Trp Tyr Thr 150 Ala Lys Thr Asp Lys Phe Val Leu Leu Lys Asn Glu Gly Leu Leu Asp 170 165 Val Ser Phe Met Leu Asn Ala Cys Tyr Asp Ile Thr Thr Glu Lys Met 180 Pro Phe Ser Pro Tyr Ile Cys Ala Gly Ile Gly Thr Asp Leu Ile Ser 200 Met Phe Glu Thr Thr Gln Asn Lys Ile Ser Tyr Gln Gly Lys Leu Gly 215 210

Leu Asn Tyr Thr Ile Asn Ser Arg Val Ser Val Phe Ala Gly Gly His

Phe His Lys Val Ile Gly Asn Glu Phe Lys Gly Ile Pro Thr Leu Leu

250

230

245

Pro Asp Gly Ser Asn Ile Lys Val Gln Gln Ser Ala Thr Val Thr Leu 260 265 270

Asp Val Cys His Phe Gly Leu Glu Ile Gly Ser Arg Phe Phe 275 280 285

<210> 20

<211> 133

<212> PRT

<213> Ehrlichia canis

<400> 20

Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu Ile Ser Ser Ile 1 5 10 15

Tyr Phe Leu Pro Asn Val Ser Tyr Ser Asn Pro Val Tyr Gly Asn Ser 20 25 30

Met Tyr Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Val Pro 35 40 45

His Phe Gly Ile Phe Ser Ala Glu Glu Glu Lys Lys Lys Thr Thr Val 50 55 60

Val Tyr Gly Leu Lys Glu Asn Trp Ala Gly Asp Ala Ile Ser Ser Gln 65 70 75 80

Ser Pro Asp Asp Asn Phe Thr Ile Arg Asn Tyr Ser Phe Lys Tyr Ala 85 90 95

Ser Asn Lys Phe Leu Gly Phe Ala Val Ala Ile Gly Tyr Ser Ile Gly
100 105 110

Ser Pro Arg Ile Glu Val Glu Met Ser Tyr Glu Ala Phe Asp Val Lys 115 120 125

Asn Gln Gly Asn Asn 130

<210> 21

<211> 686

<212> DNA

<213> Ehrlichia canis

<400> 21

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tgtaaaagca tttgccctgc agaattggga ttagtatctg aagcacttgc acaacttggt 300 aataatgcag acaaattaca agtaatttt attacaattg atccaaaaaa tgatactgta 360 gaaaaattaa aagaatttca tgaacatttt gattcaagaa ttcaaaatgtt aacaggaaat 420 actgaagaca ttaatcaaat aattaaaaaat tataaaaatat atgttggaca agcagataaa 480 gatcatcaaa ttaaccattc tgcaataatg taccttattg acaaaaaagg atcatatctt 540 tcacacttca ttccagattt aaaatcacaa gaaaatcaag tagataagtt actatcttta 600 gttaagcagt atctgtaaat aaattcatgg aatacgttgg atgagtaggt tttttttagt 660 atttttagtg ctaataacat tggcat

<210> 22 <211> 618 <212> DNA

<213> Ehrlichia chaffeensis

<400> 22
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tattcctacg taacaaaaca aggcatttt caagtaagag atcataacac tcccaataca 120
aatatatcaa ataaagccag cattactact agtttttcgt tagtaaatca agatggaaat 180
acagtaaata gtcaagattt tttgggaaaa tacatgctag ttttatttgg attttcttca 240
tgtaaaagca tctgccctgc tgaattagga atagcatctg aagttctctc acagcttggt 300
aatgacacag acaagttaca agtaatttc attacaattg atccaacaaa tgatactgta 360
caaaaattaa aaacatttca tgaacattt gatcctagaa ttcaaatgct aacaggcagt 420
gcagaagata ttgaaaaaat aataaaaaat tacaaaatat atgttggaca agcagataaa 480
gataatcaaa ttgatcactc tgccataatg tacattatcg ataaaaaagg agaatacatt 540
tcacactttt ctccagattt aaaatcaaca gaaaatcaag tagataagtt actatctata 600
ataaaacaat atctctaa

<210> 23 <211> 205 <212> PRT

<213 > Ehrlichia canis

<400> 23
Met Lys Ala Ile Lys Phe Ile Leu Asn Val Cys Leu Leu Phe Ala Ala
1 5 10 15

Ile	Phe	Leu	Gly	Tyr	Ser	Tyr	Ile	Thr	Lys	Gln	Gly	Ile	Phe	Gln	Thr
			20					25					30		

Lys His His Asp Thr Pro Asn Thr Thr Ile Pro Asn Glu Asp Gly Ile
35 40 45

Gln Ser Ser Phe Ser Leu Ile Asn Gln Asp Gly Lys Thr Val Thr Ser 50 55 60

Gln Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ala 65 70 75 80

Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Leu Val Ser Glu Ala Leu 85 90 95

Ala Gln Leu Gly Asn Asn Ala Asp Lys Leu Gln Val Ile Phe Ile Thr

Ile Asp Pro Lys Asn Asp Thr Val Glu Lys Leu Lys Glu Phe His Glu
115 120 125

His Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Thr Glu Asp Ile 130 135 140

Asp His Gln Ile Asn His Ser Ala Ile Met Tyr Leu Ile Asp Lys Lys 165 170 175

Gly Ser Tyr Leu Ser His Phe Ile Pro Asp Leu Lys Ser Gln Glu Asn 180 185 190

Gln Val Asp Lys Leu Leu Ser Leu Val Lys Gln Tyr Leu 195 200 205

<210> 24

<211> 205

<212> PRT

<213> Ehrlichia chaffeensis

<400> 24

Met Lys Val Ile Lys Phe Ile Leu Asn Ile Cys Leu Leu Phe Ala Ala 1 5 10 15

Ile Phe Leu Gly Tyr Ser Tyr Val Thr Lys Gln Gly Ile Phe Gln Val 20 25 30

Arg Asp His Asn Thr Pro Asn Thr Asn Ile Ser Asn Lys Ala Ser Ile
35 40 45

Thr Thr Ser Phe Ser Leu Val Asn Gln Asp Gly Asn Thr Val Asn Ser 50 55 60

WO 00/65063 PCT/US00/10886

22

Gln Asp Phe Leu Gly Lys Tyr Met Leu Val Leu Phe Gly Phe Ser Ser

Cys Lys Ser Ile Cys Prc Ala Glu Leu Gly Ile Ala Ser Glu Val Leu 8.5

Ser Glm Leu Gly Asn Asp Thr Asp Lys Leu Glm Val Ile Phe Ile Thr 100

Ile Asp Pro Thr Asn Asp Thr Val Gln Lys Leu Lys Thr Phe His Glu 120

His Phe Asp Pro Arg Ile Gln Met Leu Thr Gly Ser Ala Glu Asp Ile 135

Glu Lys Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys 150 155 145

Asp Asn Gln Ile Asp His Ser Ala Ile Met Tyr Ile Ile Asp Lys 170 165

Gly Glu Tyr Ile Ser His Phe Ser Pro Asp Leu Lys Ser Thr Glu Asn 180

Gln Val Asp Lys Leu Leu Ser Ile Ile Lys Gln Tyr Leu 200 195

<210> 25

<211> 618

<212> DNA

<213> Cowdria ruminantium

<220>

<221> CDS

<222> (1)..(615)

<400> 25

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att tit tig gga tat tot tac ata aca aaa caa ggt ata tic caa cca Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Pro 20

aaa tta cac gac tot oot gat gtt aat ata tog aac aaa gog gat ata Lys Leu His Asp Ser Prc Asp Val Asn Ile Ser Asn Lys Ala Asp Ile

aat act age tit age tia att aat cag gat ggt att acg ata tet agt Asn Thr Ser Phe Ser Leu Ile Asn Gln Asp Gly Ile Thr Ile Ser Ser

aaa gad tto ott gga aaa cat atg tta gto ott ttt ggg ttt tot tot

Lys 65	Asp	Phe	Leu	Gly	Lys 70	His	Met	Leu	Val	Leu 75		Gly	Phe	e Ser	Ser 80	
-										Leu	_				cta Leu	288
-					_			_			_	_		ata Ile		336
	_				_		_	-						cac His		384
							_						_	gct Ala		432
			_					_		_				gac Asp		480
_									_			-	_	aag Lys 175		528
							_		-		_			gag Glu		576
		_			ctt Leu				_	_			taa			618
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Met)> 26 Lys		Ile	-	Phe	Ile	Leu	Asn		Cys	Leu	Leu	Phe	Ala	Ala	
l Ile	Phe	Leu	Glv	5 Tvr	Ser	-د ، کیل	Tle	Thr	10	Gln	Glv	Tìe	Phe	15 Gln	Pro	
			20	- / -		- 2 -		25	2,0	02			30	0111		
Lys	Leu	His 35	Asp	Ser	Pro	Asp	Val 40	Asn	Ile	Ser	Asn	Lys 45	Ala	Asp	Ile	
Asn	Thr	Ser	Phe	Ser	Leu	Ile	Asn	Gln	qsA	Gly	Ile	Thr	Ile	Ser	Ser	

Lys Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ser Cys Lys Thr Ile Cys Pro Met Glu Leu Gly Leu Ala Ser Thr Ile Leu 90 85 Asp Gln Leu Gly Asn Glu Ser Asp Lys Leu Gln Val Val Phe Ile Thr 105 Ile Asp Pro Thr Lys Asp Thr Val Glu Thr Leu Lys Glu Phe His Lys 120 Asn Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Ile Glu Ala Ile 135 Asn Gln Ile Val Glr Gly Tyr Lys Val Tyr Val Gly Gln Pro Asp Asn 155 150 145 Asp Asn Gln Ile Asn His Ser Gly Ile Met Tyr Ile Val Asp Lys Lys 17C Gly Glu Tyr Leu Thr His Phe Val Pro Asp Leu Lys Ser Lys Glu Pro 185 180 Gln Val Asp Lys Leu Leu Ser Leu Ile Lys Gln Tyr Leu 200 <210> 27 <211> 981 <212> DNA <213> Cowdria ruminantium <220> <221> CDS <222> (1)..(978) <400> 27 atg aag aaa ata ttg gtt acg ttt tta gtt gtt gtt aat gtg ttt tgt Met Lys Lys Ile Leu Val Thr Phe Leu Val Val Val Asn Val Phe Cys aat get gee att get tea aet gae tea tea gaa gat aaa eag tat att Asn Ala Ala Ile Ala Ser Thr Asp Ser Ser Glu Asp Lys Gln Tyr Ile 20 tta att ggt act ggt tot atg act gga gta tat tat cot ata gga ggt Leu Ile Gly Thr Gly Ser Met Thr Gly Val Tyr Tyr Pro Ile Gly Gly 3.5 ago ata tgt agg ttt att goa tot gat tat ggt aat gat aat aac ago Ser Ile Cys Arg Phe Ile Ala Ser Asp Tyr Gly Asn Asp Asn Asn Ser 50 5.5

ata Ile 65	gtt Val	tgt Cys	tct Ser	ata Ile	tct Ser 70	tct Ser	aca Thr	act Thr	ggt Gly	agc Ser 75	gta Val	tat Tyr	aat Asn	ctt Leu	aat Asn 80	240
tct Ser	atg Met	cgt Arg	tat Tyr	gca Ala 85	aat Asn	atg Met	gat Asp	ata Ile	ggt 90	att	att	caa Gln	tct Ser	gat Asp 95	tta Leu	288
gag Glu	tac Tyr	tat Tyr	gca Ala 100	tat Tyr	aat Asn	ggt Gly	att Ile	ggt Gly 105	tta Leu	tat Tyr	gaa Glu	aaa Lys	atg Met 110	cca Pro	gca Ala	33€
atg Met	agg Arg	cat His 115	cta Leu	aga Arg	ata Ile	tta Leu	tct Ser 120	tca Ser	tta Leu	cat His	aaa Lys	gaa Glu 125	tat Tyr	ctt Leu	aca Thr	384
att Ile	gtt Val 130	gtt Val	agg Arg	gcg Ala	aat Asn	tct Ser 135	aat Asn	ata Ile	tca Ser	gtt Val	att Ile 140	gat Asp	gat Asp	ata Ile	aaa Lys	432
ggc Gly 145	aaa Lys	aga Arg	gtt Val	aat Asn	att Ile 150	ggt Gly	agt Ser	cct Pro	ggt Gly	act Thr 155	ggt Gly	gta Val	aga Arg	ata Ile	gca Ala 160	48C
atg Met	tta Leu	aaa Lys	ttg Leu	tta Leu 165	aat Asn	gaa Glu	aaa Lys	gga Gly	tgg Trp 170	gga Gly	aga Arg	aaa Lys	gat Asp	ttt Phe 175	gct Ala	528
gtt Val	atg Met	gca Ala	gaa Glu 180	tta Leu	aaa Lys	tca Ser	tca Ser	gag Glu 185	caa Gln	gct Ala	caa Gln	gca Ala	tta Leu 190	tgt Cys	gat Asp	576
aat Asn	aaa Lys	att Ile 195	Asp	gtg Val	atg Met	gta Val	gat Asp 200	gtt Val	gtt Val	gga Gly	cat His	ect Pro 205	aat Asn	gct Ala	gca Ala	624
att Ile	caa Gln 210	Glu	gca Ala	gca Ala	gca Ala	act Thr 215	tgt Cys	gat Asp	ata Ile	aaa Lys	ttt Phe 220	att Ile	tct Ser	tta Leu	gat Asp	672
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85

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WO 00/65063

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WO 00/65063

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WO 00/65063 PCT/US00/10886

33

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Asp Ser Leu Ile Lys 145

(19) World Intellectual Property Organization International Bureau



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Published:

With international search report.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



/e5063 */*

(54) Title: NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

(57) Abstract: Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

PCT/US 00/10886

A. CLASSIFICATION OF SUBJECT MATTER
1PC 7 C12N15/31 C07K14/29 A61K39/02 C12Q1/68 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) BIOSIS C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ° Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. WO 98 16554 A (UNIV FLORIDA) 23 April 1998 (1998-04-23) Х 1-24 the whole document Х BOWIE MICHAEL V ET AL: "Potential value of major antigenic protein 2 for 7-13 serological diagnosis of heartwater and 21-24 related Ehrlichial infections." CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, vol. 6, no. 2, March 1999 (1999-03), pages 209-215, XP000939015 ISSN: 1071-412X the whole document -/--X Further documents are listed in the continuation of box C. X Patent family members are listed in annex. Special categories of cited documents: T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance invention earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) involve an inventive step when the document is taken alone " document of particular relevance; the claimed invention cannot be considered to involve an inventive step when t document is combined with one or more other such docu "O" document referring to an oral disclosure, use, exhibition or other means ments, such combination being obvious to a person skilled in the art document published prior to the international filing date but "&" document member of the same patent family later than the priority date claimed Date of the actual completion of the international search Date of mailing of the international search report 2 2. 12. 2000 5 September 2000 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nt, ANDRES S.M. Fax: (+31-70) 340-3016

Form PCT/ISA/210 (second sheet) (July 1992)

Intern al Application No PCT/US 00/10886

	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	Delever
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NYIKA A ET AL.: "A DNA vaccine protects mice against the rickettsial agent Cowdria ruminantium." PARASITE IMMUNOLOGY (OXFORD), vol. 20, no. 3, March 1998 (1998-03), pages 111-119, XP000939081 ISSN: 0141-9838 the whole document	1-4, 6-14, 17-19
х	MAHAN S M ET AL: "Molecular cloning of a gene encoding the immunogenic 21 kDa protein of Cowdria ruminantium." MICROBIOLOGY (READING), vol. 140, no. 8, 1994, pages 2135-2142, XP000939016 the whole document	1-4, 7-13, 21-24

International application No. PCT/US 00/10886

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: See FURTHER INFORMATION sheet PCT/ISA/210
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-24
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 10 to 19 are directed to a method of treatment of the human/animal body, and claim 20 (as far as an in vivo method is concerned) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

- 1. Claims: 1-24
 - 1.1. Claims: 1-2,6-7,10-11,17-19,21-22 (all partially)
 A composition comprising a polynucleotide encoding an antigen from Rickettsia spp. and methods for using it in protection of a host against a disease or death, or in diagnostic.
 - 1.2. Claims: 1-4,6-13,17-24 (all partially), and claims 5, 15 (totally)

Compositions comprising SEQ IDs 3,4; 7,14; 8,15; 9,16; 10,17; 11,18 and 22,24 (corresponding to the MAP1, VSA1 to VSA5 and MAP2 antigens from Ehrlichia chaffeensis) and methods for using them in protection of a host against a disease or death, or in diagnostic.

- 1.3. Claims: 1-4,6-13,17-24 (all partially)
 Compositions comprising SEQ IDs 12,19; 13,20 and 21,23
 (corresponding to the VSA1, VSA2 and MAP2 antigens
 from Ehrlichia canis) and methods for using them in
 protection of a host against a disease or death, or in
 diagnostic.
- 1.4. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 16 (totally)

A compositions comprising SEQ IDs 4 and 5 (corresponding to the MSP-4 antigen from Anaplasma marginale) and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.5. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 14 (totally)

Compositions comprising SEQ IDs 1,2 and 25,26 (corresponding to the antigens MAP1 and MAP2 from Cowdria ruminantium) and methods for using them in protection of a host against a disease or death, or in diagnostic.

2. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 27 and 28 (corresponding to the 1hworf3 antigen from Cowdria ruminantium) and methods

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

for using it in protection of a host against a disease or death, or in diagnostic.

3. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 29 and 30 (corresponding to the 4hworf1 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

4. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 31 and 32 (corresponding to the 18hworf1 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

5. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 33 and 34 (corresponding to the 3gdorf3 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

ormation on patent family members

Interr ial Application No
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(54) Title: NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

(57) Abstract: Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

PCT/US00/10886 WO 00/65063

DESCRIPTION

NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

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This invention was made with government support under USAID Grant No. LAG-1328-G-00-3030-00. The government has certain rights in this invention.

Technical Field

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This invention relates to nucleic acid vaccines for rickettsial diseases of animals. including humans.

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to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus. Rickettsia prowazekii, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, e.g.. Rickettsia rickettsii and Rickettsia conorii, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe

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have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

Heartwater is another infectious disease caused by a rickettsial pathogen, namely Cowdria ruminantium, and is transmitted by ticks of the genus Amblyomma. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America.

Ehrlichiae have been described. Ehrlichiae infect leukocytes and endothelial cells of many

different mammalian species, some of them causing serious human and veterinary diseases. Over 400 cases of human ehrlichiosis, including some fatalities, caused by Ehrlichia chaffeensis

The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors

3NSDOCID: <WO 0065063A3 IA> In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenical treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] *J. Infect. Dis.* 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant *R. conorii* protein were partially protected even against *R. rickettsii* (Vishwanath, S., G. McDonald, N. Watkins [1990] Infect. Immun. 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes, e.g., protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] Advances in Vet. Sci. and Comp. Med. 27:427-480; Du Plessis. Plessis, J.L. [1970] Onderstepoort J. Vet. Res. 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against *R. prowazekii* and *R. rickettsii*. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective," In *Rickettsia and Rickettsial Diseases*, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood. This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs

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needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinates. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

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A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsia. The genes encoding the antigens and that can be employed to recombinantly produce those antigen have also been identified and sequenced. Certain protective antigens identified for *R. rickettsii*, *R. conorii*, and *R. prowazekii* (e.g., rOmpA and rOmpB) are large (>100 kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

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Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue. WHO meeting on nucleic acid vaccines [1994] *Vaccine* 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CTL). T-helper 1. and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donelly, S. Parker *et al* [1993] *Science* 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specificCTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the *Plasmodium yoelii* circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] *Froc. Natl. Acad. Sci. USA* 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox. G., T. Zamb, L. Babiuk [1993] *J. Virol.* 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has

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not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

Brief Summary of the Invention

Disclosed and claimed here are novel vaccines for conferring immunity to rickettsia infection, including *Cowdria ruminantium* causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to confer immunity in a susceptible host. Also disclosed are novel materials and methods for diagnosing infections by *Ehrlichia* in humans or animals.

One aspect of the subject invention concerns a nucleic acid, e.g., DNA or mRNA. vaccine containing the major antigenic protein 1 gene (MAP1) or the major antigenic protein 2 gene (MAP2) of rickettsial pathogens. In one embodiment, the nucleic acid vaccines can be driven by the human cytomegalovirus(HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention, immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in *in vitro* lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia. Accordingly, the subject invention concerns the discovery that DNA vaccines can induce protective immunity against rickettsial disease or death resulting therefrom.

The subject invention further concerns the genes designated Cowdria ruminantium map 2. Cowdria ruminantium 1hworf3. Cowdria ruminantium 4hworf1. Cowdria ruminantium 18hworf1. and Cowdria ruminantium 3gdorf3 and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine.

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Brief Description of the Drawings

Figures 1A-1C show a comparison of the amino acid sequences from alignment of the three rickettsial proteins, namely, Cowdria ruminantium (C.r.), Ehrlichia chaffeensis (E.c.), and Anaplasma marginale (A.m.).

Figures 2A-2C shows the DNA sequence of the 28 kDa gene locus cloned from *E. chaffeensis* (Fig. 2A-2B) and *E. canis* (Fig. 2C). One letter amino acid codes for the deduced protein sequences are presented below the nucleotide sequence. The proposed sigma-70-like promoter sequences (38) are presented in bold and underlined text as -10 and -35 (consensus -35 and -10 sequences are TTGACA and TATAAT, respectively). Similarly, consensus ribosomal binding sites and transcription terminator sequences (bold letter sequence) are identified. G-rich regions identified in the *E. chaffeensis* sequence are underlined. The conserved sequences from within the coding regions selected for RT-PCR assay are identified with italics and underlined

Figure 3A shows the complete sequence of the MAP2 homolog of *Ehrlichia canis*. The arrow (\rightarrow) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

Figure 3B shows the complete sequence of the MAP2 homolog of *Ehrlichia chaffeensis*. The arrow (\rightarrow) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

Brief Description of the Sequences

SEQ ID NO. 1 is the coding sequence of the MAP1 gene from *Cowdria ruminantium* (Highway isolate).

SEQ ID NO. 2 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1

SEO ID NO. 3 is the coding sequence of the MAP1 gene from Ehrlichia chaffeensis.

SEQ ID NO. 4 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

SEQ ID NO. 5 is the Anaplasma marginale MSP4 gene coding sequence.

SEO ID NO. 6 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

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SEQ ID NO. 7 is a	partial coding	sequence	of the	VSA1	gene f	rom	Ehrlichia
chaffeensis. also shown in Figu	res 2A-2B.						

SEQ ID NO. 8 is the coding sequence of the VSA2 gene from *Ehrlichia chaffeensis*. also shown in Figures 2A-2B.

SEQ ID NO. 9 is the coding sequence of the VSA3 gene from *Ehrlichia chaffeensis*. also shown in Figures 2A-2B.

SEQ ID NO. 10 is the coding sequence of the VSA4 gene from *Ehrlichia chaffeensis*. also shown in Figures 2A-2B.

SEQ ID NO. 11 is a partial coding sequence of the VSA5 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 12 is the coding sequence of the VSA1 gene from *Ehrlichia canis*, also shown in Figure 2C.

SEQ ID NO. 13 is a partial coding sequence of the VSA2 gene from *Ehrlichia canis*, also shown in Figure 2C.

SEQ ID NO. 14 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 7. also shown in Figures 2A-2B.

SEQ ID NO. 15 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 8. also shown in Figures 2A-2B.

SEQ ID NO. 16 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 9. also shown in Figures 2A-2B.

SEQ ID NO. 17 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 10. also shown in Figures 2A-2B.

SEQ ID NO. 18 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 11. also shown in Figures 2A-2B.

SEQ ID NO. 19 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 12. also shown in Figure 2C.

SEQ ID NO. 20 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 13. also shown in Figure 2C.

SEQ ID NO. 21 is the coding sequence of the MAP2 gene from *Ehrlichia canis*, also shown in Figure 3A.

SEQ ID NO. 22 is the coding sequence of the MAP2 gene from *Ehrlichia chaffeensis*. also shown in Figure 3B.

SEQ ID NO. 23 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 21. also shown in Figure 3A.

WO 00/65063 PCT/US00/10886

SEQ ID NO. 24 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 22. also shown in Figure 3B.

SEQ ID NO. 25 is the coding sequence of the map2 gene from Cowdria ruminantium.

SEQ ID NO. 26 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 25.

SEQ ID NO. 27 is the coding sequence of the ihworf3 gene from Cowdria ruminantium.

SEQ ID NO. 28 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 27.

SEQ ID NO. 29 is the coding sequence of the 4hworfl gene from Cowdria ruminantium.

SEQ ID NO. 30 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 29.

SEQ ID NO. 31 is the coding sequence of the 18hworfl gene from Cowdria ruminantium.

SEQ ID NO. 32 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 31.

SEQ ID NO. 33 is the coding sequence of the 3gdorf3 gene from Cowdria ruminantium.

SEQ ID NO. 34 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 33.

Detailed Disclosure of the Invention

In one embodiment, the subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response.

According to the subject invention, recombinant DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where an immune response is induced. Prokaryotic signal sequences may be deleted from the nucleic acid encoding an antigen of interest. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal. Sec. for example, Wolff, J.A., J.J. Ludike, G. Acsadi, P. Williams, A. Jani (1992) *Hum. Mol. Genet.* 1:363. A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

In one embodiment, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been

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shown to be protective against *Cowdria ruminantium*, the causative agent of heartwater in domestic ruminants. Accordingly, nucleotide sequences of rickettsial genes, as described herein, can be used as nucleic acid vaccines against human and animal rickettsial diseases.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine. In another embodiment of the invention, the polynucleotide vaccine is administered in the form of a "cocktail" which contains at least two of the nucleic acid vaccines of the subject invention. The "cocktail" may be administered in conjunction with an antigen or an antigen booster as described above.

The MAP1 gene, which can be used to obtain this protection, is also present in other rickettsiae including *Anaplasma marginale*, *Ehrlichia canis*, and in a causative agent of human ehrlichiosis, *Ehrlichia chaffeensis* (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] *Infect. Immun.* 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain *Rickettsia* spp. MAP1-like genes from *Ehrlichia chaffeensis* and *Ehrlichia canis* have now been cloned and sequenced. These MAP-1 homologs are also referred to herein as Variable Surface Antigen (VSA) genes.

The present invention also concerns polynucleotides encoding MAP2 or MAP2 homologs from *Ehrlichia canis* and *Ehrlichia chaffeensis*. MAP2 polynucleotide sequences of the invention can be used as vaccine compositions and in diagnostic assays. The polynucleotides can also be used to produce the MAP2 polypeptides encoded thereby.

The subject invention further concerns the genes designated Cowdria ruminantium map 2. Cowdria ruminantium 1hworf3. Cowdria ruminantium 4hworf1. Cowdria ruminantium 18hworf1, and Cowdria ruminantium 3gdorf3 and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid vaccine vectors (plasmids), which are commercially available (e.g., Vical, San Diego, CA). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*. Mack Publishing Company, Easton, PA.

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The subject invention also concerns polypeptides encoded by the subject polynucleotides. Specifically exemplified are the polypeptides encoded by the MAP-1 and VSA genes of *C. rumimontium*, *E. chaffeensis*. *E. canis* and the MP4 gene of *Anaplasma marginale*. Polypeptides uncoded by *E. chaffeensis* and *E. canis* MAP2 genes are also exemplified herein.

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Also encompassed within the scope of the present invention are fragments and variants of the exemplified polynucleotides and polypeptides. Fragments would include, for example, portions of the exemplified sequences wherein procaryotic signal sequences have been removed. Examples of the removal of such sequences are given in Example 3. Variants include polynucleotides and/or polypeptides having base or amino acid additions, deletions and substitutions in the sequence of the subject molecule so long as those variants have substantially the same activity or serologic reactivity as the native molecules. Also included are allelic variants of the subject polynucleotides. The polypeptides of the present invention can be used to raise antibodies that are reactive with the polypeptides disclosed herein. The polypeptides and polynucleotides can also be used as molecular weight markers.

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Another aspect of the subject invention concerns antibodies reactive with MAP-1 and MAP2 polypeptides disclosed herein. Antibodies can be monoclonal or polyclonal and can be produced using standard techniques known in the art. Antibodies of the invention can be used in diagnostic and therapeutic applications.

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In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus (HCMV) enhancer-promoter. In a specific example, this vaccine was injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50 µl/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCL1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and C. ruminantium antigens in in vitro lymphocyte proliferation tests. These proliferating cells from mice immunized with VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100 µg/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of C. ruminantium. Survival rates of 0% to 3% (1/144 survivors/totalin all control groups) were recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, in a specific embodiment, the subject invention

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concerns the discovery that the gene encoding the MAP1 protein induces protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, e.g., whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*. Stockton Press, New York, NY., pp. 169-170.

Examples of various stringency conditions are provided herein. Hybridization of

immobilized DNA on Southern blots with 32P-labeled gene-specific probes can be performed by standard methods (Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York.). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded

temperature (Tm) of the DNA hybrid in 6X SSPE. 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml

DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting

denatured DNA. The melting temperature is described by the following formula (Beltz et al.

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et al. [1983] Methods of Enzymology, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

 $Tm=81.5\,^{\circ}C+16.6\,Log[Na+]+0.41(\%G+C)-0.61(\%formamide)-600/length\ of\ duplex\ in\ base\ pairs.$

Washes are typically carried out as follows:

- (1) twice at room temperature for 15 minutes in 1X SSPE. 0.1% SDS (low stringency wash):
- (2) once at Tm-20°C for 15 minutes in 0.2X SSPE. 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization can be carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes can be determined by the following formula:

Tm (°C)=2(number T/A base pairs) +4(number G/C base pairs) (Suggs et al. [1981] ICN-UCLA Symp. Dev. Biol. Using Purified Genes. D.D. Brown [ed.], Academic Press. New York, 23:683-693).

Washes can be carried out as follows:

- (1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash:
- (2) once at the hybridization temperature for 15 minutes in 1X SSPE. 0.1% SDS (moderate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low:

1 or 2X SSPE, room temperature

Low:

1 or 2X SSPE. 42°C

Moderate:

0.2X or 1X SSPE. 65°C

High:

0.1X SSPE. 65°C.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given

polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal3* 1 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, New York: Wei *et al.* (1983) *J. Biol. Chem.* 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

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Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia *Cowdria ruminantium*. The vaccine construct tested was the MAP1 gene of *C. ruminantium* inserted into plasmid VCL1010 (Vical, San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 μg VCL1010/MAP1 DNA (V/M in Table 1 below), or 100, 50 μg VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of *C. ruminantium* and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

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	Table 1												
	100 μg V/M	75 μg V/M	50 μg V/M	25 μg V/M	100 μg V	50 μg V	Sal.						
Survived	5	7	5	3	0	0	0						
Died	3	1	3	5	8	8	8_						

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The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 µg VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection, 30 mice/group were challenged with 30LD50 of *C. ruminantium* and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

			Table 2			
	V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.
Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls (p<0.05).

Again, as summarized in Table 2, the VCL1010/MAP1 DNA vaccine increased the numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to *C. ruminantium* in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

Example 2 – Cloning and sequence analysis of MAP1 homologue genes of E. chaffeensis and E. cunis

Genes homologous to the major surface protein of *C. rummantium* MAP1 were cloned from *E. chaffeensis* and *E. canis* by using PCR cloning strategies. The cloned segments represent a 4.6 kb genomic locus of *E. chaffeensis* and a 1.6 kb locus of *E. canis*. DNA sequence generated from these clones was assembled and is presented along with the deduced amino acid

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sequence in Figures 2A-2B (SEQ ID NOs. 7-11 and 14-18) and Figure 2C (SEQ ID NOs. 12-13 and 19-20). Significant features of the DNA include five very similar but nonidentical open reading frames (ORFs) for *E. chaffeensis* and two very similar, nonidentical ORFs for the *E. canis* cloned locus. The ORFs for both *Ehrlichia* spp. are separated by noncoding sequences ranging from 264 to 310 base pairs. The noncoding sequences have a higher A+T content (71.6% for *E. chaffeensis* and 76.1% for *E. canis*) than do the coding sequences (63.5% for *E. chaffeensis* and 68.0% for *E. canis*). A G-rich region -200 bases upstream from the initiation codon, sigma-70-like promoter sequences, putative ribosome binding sites (RBS), termination codons, and palindromic sequences near the termination codons are found in each of the *E. chaffeensis* noncoding sequences. The *E. canis* noncoding sequence has the same feature except for the G-rich region (Figure 2C: SEQ ID NOs. 12-13 and 19-20).

Sequence comparisons of the ORFs at the nucleotide and translated amino acid levels revealed a high degree of similarity between them. The similarity spanned the entire coding sequences, except in three regions where notable sequence variations were observed including some deletions/insertions(Variable Regions I, II and III). Despite the similarities no two ORFs are identical. The cloned ORF 2, 3 and 4 of E. chaffeensis have complete coding sequences. The ORF1 is a partial gene having only 143 amino acids at the C-terminus whereas the ORF5 is nearly complete but lacks 5-7 amino acids and a termination codon. The cloned ORF2 of E. canis also is a partial gene lacking a part of the C-terminal sequence. The overall similarity between different ORFs at the amino acid level is 56.0% to 85.4% for E. chaffeensis. whereas for E. canis it is 53.3%. The similarity of E. chaffeensis ORFs to the MAP1 coding sequences reported for C. ruminantium isolates ranged from 55.5% to 66.7%, while for E. canis to C. ruminantium it is 48.5% to 54.2%. Due to their high degree of similarity to MAP1 surface antigen genes of C. ruminantium and since they are nonidentical to each other, the E. chaffeensis and E. canis ORFs are referred to herein as putative Variable Surface Antigen (VSA) genes. The apparent molecular masses of the predicted mature proteins of E. chaffeensis were 28.75 kDa for VSA2, 27.78 for VSA3, and 27.95 for VSA4, while E. canis VSA1 was slightly higher at 29.03 kDa. The first 25 amino acids in each VSA coding sequence were eliminated when calculating the protein size since they markedly resembled the signal sequence of C ruminantium MAP1 and presumably would be absent from the mature protein.

The amino acid sequence derived from the cloned *E. chaffeensis* MAP1-like gene. and alignment with the corresponding genes of *C. ruminantium* and *A. marginale* is shown in Figure 1.

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Example 3

A further aspect of the subject invention are five additional genes which give protection when formatted as DNA vaccines. These genes are Cowdria ruminantium map 2. Cowdria ruminantium 1hworf3. Cowdria ruminantium 4hworf1. Cowdria ruminantium 18hworf1. and Cowdria ruminantium 3gdorf3. The DNA and translated amino acid sequences of these five genes are shown in SEQ ID NOS. 25-34.

There is published information showing that gene homologs of all five genes are present in other bacteria. For example, a homolog of *map2* is present in *Anaplasma marginale*, a homolog of *Ihworf3* is present in *Brucella abortus*, homologs of *Ihworf1* are present in *Pseudomonas aeruginosa* and *Coxiella burnetii*, and homologs of *I8hworf1* are present in *Coxiella burnetii* and *Rickettsia prowazekii*. This can be revealed by a search of DNA and protein databases with standard search algorithms such as "Blast". Based on the protective ability of these genes against *Cowdria ruminantium* and their presence in other bacterial pathogens, the subject invention further concerns the use of these genes, their gene products, and the genes and gene products of the homologs as vaccines against bacteria. This includes their use as DNA or nucleic acid vaccines or when formulated in vaccines employing other methods of delivery *e.g.*, recombinant proteins or synthetic peptides in adjuvants, recombinant live vector delivery systems such as vaccinia (or other live viruses) or *Salmonella* (or other live bacteria). These methods of delivery are standard to those familiar with the field. This also includes vaccines against heartwater disease, vaccines against rickettsial diseases in general and vaccines against other bacteria containing homologs of these genes.

Table 3 shows the protective ability of the 5 genes against death from *Cowdria ruminantium* challenge in mice. Genes were inserted into VR1012 according to the manufacturers instructions(Vical, San Diego) and challenge studies were conducted as described in Example 1. N-terminal sequences which putatively encoded prokaryotic signal peptides were deleted because of the potential for their affects on expression and and immune responses in eukaryotic expression systems or challenged animals. The inserts were as follows: map2. SEQ ID NO. 25, beginning at base 46: 18hworf1, SEQ ID NO. 31, beginning at base 67: 3gdorf3, SEQ ID NO. 33, beginning at base 79: lhworf3, SEQ ID NO. 27, beginning at base 76: and 4hworf1, SEQ ID NO. 29, beginning at base 58.

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	Table 3													
DNA Construct	MWT		Survival Rate											
	Size	Vacci	nated	Con	trol	P value								
TMMAP 2	21 kd	9/28*	32%	0/29	00.0	0.004								
MB18HWORF1	28 kd	10/30*	3300	1/27	40%	0.021								
AM3GDORF3	16 kd	7/26	27°⁄o	1/27	40%	0.060								
TM1HWORF3	36 kd	8/29	28%	2/30	7%	0.093								
TM4HWORF1	19 kd	10/30*	33%	2/30	7%	0.054								

Control - VR1012 DNA vector plasmid only

*Statistically significant difference (Fisher's Exact test)

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

<u>Claims</u>

1	1. A composition comprising a polynucleotide which encodes a polypeptide having the
2	characteristic of eliciting an immune response protective against disease or death caused by a
3	rickettsial pathogen.
1	2. The composition, according to claim 1, wherein said rickettsial pathogen is selected
2	from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and Cowdria spp.
1	3. The composition, according to claim 1, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6.
3	SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ
4	ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30, SEQ ID NO. 32. SEQ ID NO. 34. homologs
5	thereof, and immunogenic fragments thereof.
1	4. The composition, according to claim 1, wherein said polynucleotide has a nucleic
2	acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.
3	5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22., SEQ
4	ID NO. 25. SEQ ID NO. 27. SEQ ID NO. 29. SEQ ID NO. 31. SEQ ID NO. 33. homologs
5	thereof, and fragments thereof which encode immunogenic polypeptides.
]	5. The composition, according to claim 4, wherein said polynucleotide has a nucleic
2	acid sequence of SEQ ID NO. 3. or a fragment thereof.
1	6. The composition, according to claim 1, wherein said polynucleotide further
2	comprises a nucleic acid vaccine vector.
1	7. The composition, according to claim 1, further comprising a pharmaceutically
2	acceptable carrier.
1	8. A polynucleotide encoding a polypeptide having an amino acid sequence selected
2	from the group consisting of SEQ ID NO. 4, SEQ ID NOS. 14-20, SEQ ID NOS. 23-24, SEQ
3	ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30. SEQ ID NO. 32. SEQ ID NO. 34, and fragments
4	thereof.

l	9. The polynucleotide according to claim 8, said polynucleotide having a nucleic acid
2	sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NOS. 7-13, SEQ ID
3	NOS. 21-22. SEQ ID NOS. 25. SEQ ID NO. 27. SEQ ID NO. 29. SEQ ID NO. 31, and SEQ ID
4	NO. 33.
	blo bost against disease or death caused by a
1	10. A method for protecting a susceptible host against disease or death caused by a
2	rickettsial pathogen, said method comprising administering an effective amount of a
3	polynucleotideencoding polypeptide having the characteristic of eliciting an immune response
4	protective against said rickettsial pathogen.
1	11. The method, according to claim 10, wherein said rickettsial pathogen is selected
2	from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and Cowdria spp.
	The state of the s
1	12. The method, according to claim 10, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6.
3	SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ
4	ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30. SEQ ID NO. 32. SEQ ID NO. 34, or homologs
5	thereof and immunogenic fragments thereof.
1	13. The method, according to claim 10, wherein said polynucleotide has a nucleic acid
2	sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5.
3	SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID
4	NO. 25. SEQ ID NO. 27. SEQ ID NO. 29. SEQ ID NO. 31. and SEQ ID NO. 33.
l	14. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
2	sequence of SEQ ID NO. 1.
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1	15. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
2	sequence of SEQ ID NO. 3.
	16. The method, according to claim 13, wherein said polynucleotide has the nucleic acid
1	
2	sequence of SEQ ID NO. 5.

17. The method, according to claim 10, wherein said nucleic acid further comprises an

2	appropriate nucleic acid vector.
1	18. The method, according to claim 10, wherein said composition further comprises a
2	pharmaceutically acceptable carrier.
1	19. The method, according to claim 10, which further comprises administration to said
2	host of said polypeptide encoded by said polypeptide.
1	20. A method for detecting, in a human or animal, antibodies associated with infection
2	by Ehrlichia, wherein said method comprises contacting a biological fluid from said human or
3	animal with a polypeptide selected from the group consisting of SEQ ID NO. 4, SEQ ID NOS.
4	14-20. SEQ ID NOS. 23-24, SEQ ID NO. 26. SEQ ID NO. 28. SEQ ID NO. 30. SEQ ID NO.
5	32, SEQ ID NO. 34, and homologs and fragments thereof.
1	21. A method of detecting the presence of rickettsial nucleic acids comprising
2	contacting a sample suspected of containing rickettsial nucleic acids with a composition
3	comprising a labeled polynucleotide which encodes a polypeptide having the characteristic of
4	eliciting an immune response protective against disease or death caused by a rickettsial
5	pathogen, allowing for the formation of a hybridization complex and detecting said label.
I	22. The composition, according to claim 21, wherein said rickettsial pathogen is
2	selected from the group consisting of Rickettsia spp., Ehrlichia spp., Anaplasma spp., and
3	Cowdria spp.
1	23. The composition according to claim 21, wherein said polypeptide has an amino acid
2	sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6.
3	SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ
4	ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and homologs
5	and immunogenic fragments thereof.
1	24. The composition, according to claim 21, wherein said polynucleotide has a nucleic
2	acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO
3	5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22. , SEQ

- 4 ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs
- 5 thereof, and fragments thereof which encode immunogenic polypeptides.

FIG. 1A

ATGAATTGCAAGAAATTTTTA——————————TCACAAGTACACTAATATCATTAGTG ATGAATTACAAAAAAGTTTCA———————————TAACAGCG—ATTGATATCATTAATA ATGAATTACAGAGAATTGTTTACAGGGGGCCTG—TCAGCAGCC—ACAGTCTGCGCCTGCT ****** ** ** ** ** ** ** ** ** ** ** **

CCCTACTTGITAGTGGGGCCGTAGTGGCATCTCCCATGAGTCACGAAGTGGCTTCTGAAG TCATTTT--TACCTGGTGTGTCCTTTTTCTGATGTAATACAGGAAGACAGCAACCCAGCAG TCCTTCTCTTACCTGGAGTATCATTTTCCGACCCAAGGCAGGTAGTGGTCA---TTAACG * *** A.m. C.Y. E.c.

GTAATTTCTACATCAGTGGAAAATACGATGCCAAGGCTTCGCCATTTTGGAGTATTCTCTG GGGGAGTAATGGGAGGTAGCTTTTACGTGGGGTGCGGCCT-ACAGCCCCAGCATTTCCTTCT GCAGTGTTTACATTAGCGCAAAATACATGCCAACTGCATCACATTTTGGTAAAATGTCAA A.m. C.r.E.c.

CTAAGGAAGAAAATACAACAGTTGGAGTGTTTGGACTGAAGCAAAATTGGGACGGAA GTTACCTCGTTCGACATGCGTGAGTCAAGCAAAGAGACCTCA--TACGTTAGAGGCTATG E.c. C.r.

GCGCAATATC--CAACTCCTCCCCAAACGA-----TGTATTCACTGTCTCAAATTATT ACAAGAGCATTGCAACGATGTGTGTGCCAGCAAACTTTTCCAAATCTGGCTACA TTAAAACACCATCAGATTCTAGCAATACTAATTCTACAATTTTTACTGAAAAAAGACTATT A.m. C.Y. E.c.

CATTTAAATATGAAAACCACGTTTTTAGGTTTTTGCAGGAGCTATTGGTTACTCAATGG CTTTCAGATATGAAAACAATCCGTTTTTAGGTTTCGCTGGAGCAATTGGGTACTCAATGA CTTTTGCCTTCTCTAAAACTTAATCACGTCTTTCGACGGCGCTGTGGGATATTCTCTGG ** **

A.m.

C.r. E.c.

FIG. 1B

ATGGACCAAGAATAGAGTTCGAAGTATCCTATGAAACTTTTGATGTAAAAAACCTAGGTG ATGGTCCAAGAATAGAGCTTGAAGTATCTTATGAACATTTGATGTAAAAAATCAAGGTA GAGGAGCCAGAGTGGAATTGGAAGCGAGCTACAGAAGGTTTGCTACTTTGGCGGACGGGC *** * *** * A.m. E.c. C.r.

GCAACTATAAAAACAACGCACACATGTACTGTGCTTTAGATACAGCAGCACAAAATAGCA ACAATTATAAGAATGAAGCACATAGATATTGTGCTCTATCCCATAACTCAGCAGCAGACA --GIGCGGAAICICIGGCAGCIAIIACCCGCG AGTACGCAAAAAGTG--

A.m.

C.r. E.c.

ACGCTAACATTACTGAGACCAATTACTTCGTAGTCAAAATTGATGAAATCACAAACACCT TGAGTAGTGCAAG---TAATAATTTTGTCTTTCTAAAAAATGAAGGATTACTTGACATAT **** E.c.4.m. C.r.

CATTAATGTTAAATGCGTGTTATGATATCATGCTTGATGGAATACCAGTTTCTCCATATG CATTIAIGCIGAACGCAIGCIAIGACGIAGIAGGCGAAGGCAIACCIITIICICCIIAIA CAGTCATGTTAAATGGCTGCTATGACGTGCTGCACACAGATTTACCTGTGTCCCCGTATG **** ** * ** * *** * **

> E.c. A.m.

C.Y.

E.c.

C.r.

TATGCGCAGGTATCGGTACTGATTTAGTATCCATGTTTGAAGCTACAAATCCTAAAATTT TATGTGCCGGGATAGGCGCAAGCTTTGTTGACATCTCTAAGCAAGTAACCACAAAAGCTGG CTTATCAAGGAAAGCTAGGCATAAGTTACTCAATTCTAATTCTGAAGCTTCTATCTTATCG CTTACCAAGGAAAGTTAGGTTTAAGCTACTCTATAAGCCCAGAAGCTTCTGTGTTTATTG CCTACAGGGGCAAGGTTGGGATTAGCTACCAGTTTACTCCGGAAATATCTTGGTGGCAG ** * *** **

A.m.

C.r. E.c.

FIG. 1C

GTGGACATTTCCATAGAGTTATAGGTAATGAATTTAAAGATATTGCTACCTTAAAAATAT GIGGGTICTACCACGGGCTATITGATGAGTCTTACAAGGACATTCCCGCACACAACAGTG GIGGGCACTITCATAAGGTAATAGGGAACGAATTTAGAGATATTCCTACTATATACCTA * * *

CTGGATCAACACTTGCAGGAAAAGGAAACTACCCTGCAATAGTAATACTGGATGTATGCC .----GCCTCAGTCAAAGCGCATATTGCTG TTACTTCAAAAACAGGAATATCTAATCCTGGCTTTGCATCAGCAACACTTGATGTTTGTC TAAAGTTCTCGGAGAAGCAAAA-

ACTACGGCTTTAACCTTGGAGCAAGATTCCTGTTCAGCTAA ACTTTGGTATAGAAATTGGAGGAAGGTTTGTATTTTAA----ACTTTGGAATAGAAATGGGAGGAAGGTTTAA------** *** *** C.r. A.m.

E.c.

C.r. E.c. A.m.

A.m.

C.r. E.c.

1 ggaatgaattcagggacatttctactcttaaagcgtttgctacaccatcatctgcagcta NEFRDISTLKAFATPSSAAT 61 ctccagacttagcaacagtaacactgagtgtgtgtcactttggagtagaacttggaggaa PDL ATVTLSVCHFGVELGGR 121 gatttaacttotaattttattattgccacatgttaaaaataatctaaacttgttttcatt 181 ettgctacaaataaataaataaatagtggcaaaaaaatgtagcaataagaagaggggggaa 241 ccaattactatctgccatatcccttactaccacttacactaaataatctgacaaatacaa 301 cagottctggagaaataaacaatatttaaattttccttacaaaaaccatttatatcttgt 361 actaaaaactagcttataacttgtttttacattgtaggtttactactgttaatttgtttt -10 421 cactatttcaqqtqtaatatgaactgcgaaaaattttttataacaactgcattaacatta MNCEKFFITTALT 481 ctaatgteettettaeetggaatateaetttetgateeagtaeaggatgaeaaeattagt LMSFLPGISLSDPVQDDNIS 541 ggtaatttetacateagtggaaagtatatgecaagegettegeatttttggagttttttet G N F Y I S G K Y H P S A S H F G V F S 601 gccaaggaagaaagaaacacaacagttggagtatttggaatagagcaagattgggataga A K E E R N T T V G V F G I E Q D W D R 661 tgtgtaatatctagaaccactttaagegatatattcaecgttccaaa<u>ttattcatttaag</u> CVISRTTLSDIFTVPNYSFK Y E N N L F S G F A G A I G Y S M D G P 781 agaatagagettgaagtatetTatgaageattegatgttaaaaateaaggtaacaattat RIELEVSYEAPDVKNQGNNY 841 aagaacgaagcacatagatattatgototgtoocatottotoggcacagagacacagata KNEAHRYYALSHLLGTETQI 901 gatggtgcaggcagtgcgtctgtctttctaataaatgaaggactacttgataaatcattt DGAGSASVFLINEGLLDKSP 961 atgctgaacgcatgttatgatgtaataagtgaaggcatacettttteteettatatatgt MLNACYDVISEGIPFSPYIC 1021 gcaggtattggtattgatttagtatccatgtttgaagctataaatcctaaaatttcttat EAI 11 KISY AGIGIDLVSHF 1081 caaggaaaattaggcttaagttaccctataagcccagaagcttctgtgtttattggtgga QGKLGLSYPISPEASVFIGG 1141 cartttcataaggtgataggaaacgaarttagagatartcctactatgatacctagtgaa H F H K V I G N E F R D I P T H I P S E 1201 teagegettgcaggaaaaggaaactaceetgcaatagtaacactggacgtgttctacttt SALAGKGNYPAIVTLDVFYF GIELGGRFNFQL 1441 aaacaattottaaatttgtottatgagaaccattgatatottatattaaaaactagotta -35 RRS -10 1561 atatgaattgcaaaaaattttttataacaactgcattagtatcactaatgtcctttctac MNCKKPFITTALVSLMSFLP 1621 ctggaatatcattttctgatccagtgcaaggtgacaatattagtggtaatttctatgtta G I S F S D P V Q G D N I S G N F Y V S 1681 gtggcaagtatatgccaagtgcttcgcatttttggcatgttttctgccaaagaagaaaaaa G K Y M P S A S H F G M P S A K E E K N 1741 atcctactgttgcattgtatggcttaaaacaagattgggaagggattagctcatcaagtc PTVALYGLKQDWEGISSSSH 1801 acaatgataatcatttcaataacaagggttattcatttaatataaatatgaaaataacccatttt SFKYENNPFL Y NDNHPNNKG FAGAIGYSMGGPRVEFEV 1921 cctatgaaacatttgacgttaaaaatcagggtaataactataaaaatgatgctcacagat YETFDVKNQGNNYKNDAHRY 1981 actgtgctttaggtcaacaagacaacagcggaatacctaaaactagtaaatacgtactgt CALGQQDNSGIPKTSKYVLL K S E G L L D I S F M L N A C Y D I I N 2101 acgagageatacetttgteteettacatatgtgeaggtgttggtActgatttaatateea ESIPLS PYICAG V G T D L I S M 2161 tatttaaagetacaaateetaaaaatttettaccaagggaagttaggtetaagttacteta FEATNP KISYQG KLG L SYSI 2221 taaacccagaagcttctgtatttattggtggacattttcataaggtgataggaaacgaat NPEASVFIGGHFHKVIGNEF 2281 ttagggacattcctactctgaaagcatttgttacgtcatcagctactccagatctagcaa RDIPTLKAFVTSSATPDLAI

FIG. 2A

2341 tagtaacactaagtgtatgtcattttggaatagaacttggaggaaggtttaacttttaat V T L S V C H F G I E L G G R F N F * 2401 tregreactgccacacgttaaaaataatctaaactgtretcattattgctacagtaaat 2461 saaaatagtggcamaagaatgtagcaatsagaaggagagagagaactaaattgctattt 2521 accatacecettattataceaettacaetaaataaettgaeaaatacaaeagettetgga 2581 aaaacaaacaacacttaaacttcctcttacaaaaaccatttataccttgtaccaaaaacta - 35 -10 2701 gtgcaatatgaattgcaaaaaattttttataacaactacattagtatcgctaatgtcott RBS M N C K K F F I T T T L V S L M S P 2761 cttacctggaatatcattttctgatgcagtacagaacgacaatgttggtggtaatttcta LPGISFSDAVQNDNVGGNFY 2821 tatcagtgggaaatatgtaccaagtgtttcacattttggcgtattctctgctaaacagga I S.G.K Y V P S V S H F G V F S A K Q E 2881 aagaaatacaacaatcggagtatttggattaaagcaagattgggatggcagcacaatatc RNTTIGVFGLKQDWDGSTIS 2941 caaaaattcccccagaaaatacatttaacgttccaaa<u>ttattcatttaaatatsaa</u>aataa KNSPENTFNVPNYSFKYENN 3001 tocatttotaggttttgcaggagetgttggttatttaatgaatggtccaagaatagagtt PPLGPAGAVGYLMNGPRIEL 3061 agaaatgtcctatgaaacatttgatgtgaaaaaccagggtaataactataagaacgatgc EMSYETFOVKNQGNNYKNDA 3121 tcacaaatattatgctttaacccataacagtgggggaaagctaagcaatgcaggtgataa H K Y Y A L T H N S G G K L S N A G D K F V F L K N E G L L D I S L M L N A C Y 3241 tgatgtaatsagtgaaggaatacctttctctctcttacatatgtgcaggtgttggtactga DVISEGIPFSPYICAGVGTD 3301 tttaatatecat<u>gtttgaagetataaaee</u>etaaaatttettatsaaggaaagttaggttt L I S M F E A I N P K I S Y Q G K L G L L I S M 3361 gagttactccataagcccagaagcttctgtttttgttggtggacattttcataaggtgat SYSISPEASVFVGGHFHKVI 3421 agggaatgaattcagagatattcctgctatgatacccagtacctcaactctcacaggtaa G N E F R D I P A M I P S T S T L T G N 3481 tcactttactatagtaacactaagtgtatgccactttggagtggaacttggaggaaggtt HFTIVTLSVCHFGVELGGRF 3541 taacttttaattttattattgccacatgttaaaaataatctaaacttgttttattattg N P: * 3601 ctgcaggtaaataaaatagtggcaaaagaatgtagcaataagaaogoogoogoogactag 3721 tattacttacctgacgtaatatattaaattttccttacaaaagttaccgatactttatac 3781 aaaaatttattotgacttgctttatatgacacttctacttgttaatttgtc -103841 actattaggttatatatgaattacaaaaaagttttcataacaagtgcattgatatcatta MNYKKVFITSALISL RBS 3901 atatettetetaeetggagtateatttteegaeeeageaggtagtggtattaaeggtaat ISSLPGVSFSDPAGSGINGN 3961 ttctacatcagtggaaaatacatgccaagtgcttcgcattttggagtattctctgctaag FY I S G K Y M P S A S H F G V F S A K 4021 gaagaaagaaatacaacagttyyagtgtttggactgaagcaaaartgggacggaagcgca E E R N T T V G V F G L K Q N W D G S A 4081 atatecaactectecceaaacgatgtatteactgteteaaa<u>ttatecatttaaatateaa</u> I S N S S P N D V F T V S N Y S FKYE NNPFLGFAGAIGYSHDGPRI 4201 gagettgaagtatettatgaaacatttgatgtaaaaaatcaaggtaacaattataagaat ELEVSYETFDVKNQGNNYKN 4261 gaageacatagatattgtgetetateeeataaeteageageagacatgagtagtgeaagt EAHRYCALSHNSAADMSS 4321 aacaattttgtctttctaaaaaatgaaggattacttgacatatcatttatgctgaacgca NNFVFLKNEGLLDISFMLNA 4391 tgctatgacgtagtaggcgaaggcatacctttttctcccttatatatgcgcaggtatcggt CYDVVGEGIPFSPYICAGIG 4441 actgatttagtatccatgtttgaagctacaaatcctaaaatttcttaccaaggaaagtta T D L V S M F E A T N P K I S Y Q G K L 4501 ggtttaagetaetetataageeeagaagettetgtgtttattggtgggeaettteataag G L S Y S I S P E A S V F I G G H F H K 456l gtaatagggaacgaatttagagatattootactataatacetactggatcaacacttgca VIGNEFRDIPTII PTGSTLA 4621 ggaaaaggaaactaccctgcaatagtaatactggatgtatgccactttggaatagaaatg G K G N Y P A I V I L D V C H F G I E M 4681 gga

FIG. 2B

```
1 tggtqtaaatatgaaatataaaaaaacttttacagtaactgcattagtattaacttc
RBS M K Y K K T F T V T A L V L L T S
 61 ctttacacattttatacctttttatagtccagcacgtgccagtacaattcacaacttcta
    FTHFIPFYSPARASTIHNFY
121 cattagtggaaaatatatgccaacagcgtcacattttggaattttttcagctaaagaaga
    I S G K Y M P T A S H F G I F S A K E E
181 acaaagttttactaaggtattagttgggttagatcaacgattatcacataatattataaa
    Q S F T K V L V G L D Q R L S H N I I N
241 caataatgatacagcaaagagtcttaaggttcaaaattattcatttaaatacaaaaataa
    NNDTAKSLKVQNYSFKYKNN
301 cccatttctaggatttgcaggagctattggttattcaataggcaattcaagaatagaact
    P F L G F A G A I G Y S I G N S R I E L
361 agaagtatcacatgaaatatttgatactaaaaacccaggaaacaattatttaaatgactc
    EVSHEIFDTKNPGNNYLNDS
421 toacaaatattgcgctttatctcatggaagtcacatatgcagtgatggaaatagcggaga
    H K Y C A L S H G S H I C S D G N S G D
481 ttggtacactgcaaaaactgataagtttgtacttctgaaaaatgaaggtttacttgacgt
    WYTAKTDKPVLLKNEGLLD
541 ctcatttatgttaaacgcatgttatgacataacaactgaaaaaatgcctttttcacctta
    SFMLNACYDITTEKMPFSPY
601 tatatgtgcaggtattggtactgatctcatatctatgtttgagacaacacaaaacaaaat
    I C A G I G T D L I S M F E T T Q N K I
661 atottatoaaggaaagttaggtttaaactatactataaactcaagagtttotgtttttgc
    SYQGKLGLNYTINSR
721 aggtgggcactttcataaggtaataggtaatgaatttaaaggtattcctactctattacc
    GGHFHKVIGNEFKGIPTLLP
781 tgatggatcaaacattaaagtacaacagtctgcaacagtaacattagatgtgtgccattt
    DGSNIKVQQSATVTLDVCHF
841 cgggttagagattggaagtagattttttttttaatacttctattgtacatgttaaaaata
    G L E I G S R F F F
961 aagttaaatattagaaaagtcatatgtttttcattgccattgatactcaactaaaagtag
1021 tataaatgttacttattaataattttacgtagtatattaaatttcccttacaaaagccac
1081 tagtattttatactaaaagctatactttggcttgtatttaatttgtattttactactgt
1141 taatttactttcactgtttctgtgtaaatatgaattgtaaaaaagttttcacaataagt
                            MNCKKVFTIS
                     RBS
1201 gcattgatatcatccatatacttcctacctaatgtctcatactctaacccagtatatggt
    ĀLĪSSIYFLPNVSYSNPVYG
1261 aacagtatgtatggtaatttttacatatcaggaaagtacatgccaagtgttcctcatttt
    N S M Y G N F Y I S G K Y M P S V P H F
1321 ggaattttttcagctgaagaagaagaaaaaaaagacaactgtagtatatggcttaaaagaa
    G I F S A E E E K K K T T V V Y G L K E
1381 aactgggcaggagatgcaatatctagtcaaagtccagatgataattttaccattcgaaat
    N W A G D A I S S Q S P D D N F T I R N
1441 tactcattcaagtatgcaagcaacaagtttttagggtttgcagtagctattggttactcg
Y S F K Y A S N K F L G F A V A I G Y S
1501 ataggeagtccaagaatagaagttgagatgtcttatgaagcatttgatgtaaaaaatcaa
    I Ğ S P R I E V E M S Y E A P D V K N Q
1561 ggtaacaatt
    GNN
```

FIG. 2C

1	acatg	tat	aca	tta	tag	taa	caa	aato	jtta	ccg	tat	ttt	att	cat	aag	tta	agt	aaa	ato	ET.
61	atacc	att	ctc	ttt	cac	ttt	ato	caga	aga	ctt	tta	ttt	ato	aca	aac	tca	tga	cgt	ata	ag
121	tgtca	caa	ata	aac	aca	ctç	gcaa	acto	jca a	atca	cta	cgt	aaa	act	tta	act	ctt	ctt	ttt	C
181	acaac	taa	aat	act	aat	aaa	aagt	caat	ata	gta	taa	aaa	aato	tta	agt	aac	TTG	<u>ACA</u>	taa	at
241	attac	tct	gat		GCA 10	<u>T</u> a t	gto	ctaç	gtat	ctc	tat	act	aaa	cgt	tta	tat	aat	t <u>G</u>	AG	ca
301	tatta.	ATG M		GCI A	ATC I	AA. K	ATT(F	CATA I	L L	raat n	GT(V	CTG(C	ETT <i>I</i> L	L L	ATTI F	rgc⊁ A -	AGCA A	ATA/ I	TTT F	rt L
361	TAGGG G	TAT Y	TCC S	TAT Y	TATI I	TACA	AAA) K	ACA/ Q	AGG(G	CAT <i>I</i> I	ATTI F	rcaj Q	AAC# T	AAA! K	ACAT H	rca'i H	rga'i D	TACA T	ACC:	АТ И
421	ATACT T	ACI T	ATA' I	CCP P	LAAI N	GA/ E	AGA(D	G G	TAT:	rcaj Q	ATCT S	rago s	CTTT F	rago s	L L	I	raas N	KAD1 Q	AGA(D	G G
481	GTAAA K		GTA V	ACC T	CAGC S	CAA Q	AGA: D	r T T(CCT/ L	AGG(G	K K	ACA(H	CAT(M	GTT/ L	AGT: V	rtt(STT? F	rgg) G	ATT(F	CT S
541	CTGCA A	TGI C	AAA K	AGC S	CATI I	rTG(C	CCC'	rgc/ A	AGA) E	ATT(L	G G	ATT. L	AGT/ V	ATC' S	rgaj E	AGC/ A	ACT:	rgc) A	Q Q	AC L
601	TTGGT G	raa' u	raat n	GCA A	AGA(D	CAAJ K	ATT. L	ACA Q	AGT) V	AAT' I	rtt' F	TAT' I	TAC.	AAT' I	TGA' D	rcc. P	AAA K	'AAA' N	rga' D	AT T
661	CTGTA V	GA <i>F</i> E	K K	\TT! L	AAA. K	AGAJ E	ATT F	TCA'	TGA. E	ACA' H	TTT F	TGA D	TTC. S	AAG R	AAT' I	TCA. Q	AAT(M	GTT. L	AAC. T	AG G
721	GAAAT N	'AC'I	rga <i>p</i> E	AGA(D	I I	raat N	TCA Q	AAT. I	AAT' I	TAA. K	ДДД. И	TTA Y	TAA. K	AAT. I	ATA' Y	TGT" V	rgg. G	ACA Q	AGC A	AG D
781	АТААА К	GAT D	rcat H	rcaj Q	AAT: I	raa N	CCA H	TTC' S	TGC. A	TAA I	TAA M	GTA Y	CCT L	TAT I	TGA D	CAA K	AAA K	AGG. G	ATC S	TA Y
841	ATCTT L	TC#	ACA(H	CTT(CAT?	rcc. P	AGA D	TTT. L	aaa K	ATC. S	ACA Q	AGA E	AAA N	TCA Q	AGT. V	AGA D	TAA K	GTT. L	ACT L	AT S
901	CTTTA L	GTI V	K K	GCA(GTA: Y	rct L	GTA ≠	Att	taa	taa	tta	att	<u>AAA</u>	<u>G</u> ag	aat	agt	aca	са <u>С</u>	TTT	tt
961 021	ataaa			gga	ata	cgt	tgg	atg	agt	agg	ttt	ttt	tta	gta	ttt	tta	gtg	cta	ata	.a.c

FIG. 3A

1	ggaaatctcatgtaaacgtgaaatactatattcttttttaaataccaatacaattgaata
61	caaaaaaacttttacaacttattatgtttatcttaaaaccttattttaagattccttat
121	tcacaaaataacaaaatactatttacaaaatacaccacaatttcatca
181	ctatacactttattatactacagtagatataccataaaagattttaagtaac <u>TTGACA</u> ta
241	atattaccttggta $ extbf{TAGCAT}$ atgattcagtattttatattaaaatttattatgtatt $ extbf{GGF}$
301	©cataaaATGAAAGTTATCAAATTTATATATATCTGTTTATTATTTGCAGCAATTTT M K V I K F I L N I C L L F A →A I F
361	TCTAGGATATTCCTACGTAACAAAACAAGGCATTTTTCAAGTAAGAGATCATAACACTCC L G Y S Y V T K Q G I F; Q V R D H N T P
421	CAATACAAATATATCAAATAAAGCCAGCATTACTACTAGTTTTTCGTTAGTAAATCAAGA N T N I S N K A S I T T S F S L V N Q D
481	TGGAAATACAGTAAATAGTCAAGATTTTTTGGGAAAATACATGCTAGTTTTATTTGGATT G N T V N S Q D F L G K Y M L V L F G F
541	TTCTTCATGTAAAAGCATCTGCCCTGCTGAATTAGGAATAGCATCTGAAGTTCTCTCACA
601	GCTTGGTAATGACACAGACAAGTTACAAGTAATTTTCATTACAATTGATCCAACAAATGA L G N D T D K L Q V I F I T I D P T N D
661	TACTGTACAAAAATTAAAAACATTTCATGAACATTTTGATCCTAGAATTCAAATGCTAAC T V Q K L K T F H E H F D P R I Q M L T
721	AGGCAGTGCAGAAGATATTGAAAAAATAATAAAAAATTACAAAAATATATGTTGGACAAGG G S A E D I E K I I K N Y K I Y V G Q A
781	AGATAAAGATAATCAAATTGATCACTCTGCCATAATGTACATTATCGATAAAAAAGGAGA D K D N Q I D H S A I M Y I I D K K G E
841	ATACATTTCACACTTTTCTCCAGATTTAAAATCAACAGAAAATCAAGTAGATAAGTTACT
	Y I S H F S P D L K S T E N Q V D K L L
901	ATCTATAATAAAACAATATCTCTAAtttaataattaatta <u>AAGAG</u> aatagtacaca <u>CTCT</u> S I I K Q Y L *
961 021	\underline{T} atataaattcatggatatatgtgatgggtagatttcttttggtgtttctatcgctaatta

FIG. 3B

WO 00/65063 PCT/US00/10886

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  Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Val Ile Gln Glu Asp Ser
               20
  aac cca gca ggc agt gtt tac att agc gca aaa tac atg cca act gca
                                                                     144
  Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Lys Tyr Met Pro Thr Ala
           35
  tca cat ttt ggt aaa atg tca atc aaa gaa gat tca aaa aat act caa
                                                                     192
  Ser His Phe Gly Lys Met Ser Ile Lys Glu Asp Ser Lys Asn Thr Gln
                            55
       50
  acg gta ttt ggt cta aaa aaa gat tgg gat ggc gtt aaa aca cca tca
                                                                      240
  Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gly Val Lys Thr Pro Ser
                        70
  gat tot ago aat act aat tot aca att tit act gaa aaa gao tat tot
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tac Tyr	tca Ser	atg Met 115	aat Asn	gga Gly	cca Pro	aga Arg	ata Ile 120	gag Glu	ttc Phe	gaa Glu	gta Val	tcc Ser 125	tat Tyr	gaa Glu	act Thr	384
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tac Tyr 145	tgt Cys	gct Ala	tta Leu	gat Asp	aca Thr 150	gca Ala	gca Ala	caa Gln	aat Asn	agc Ser 155	act Thr	aat Asn	ggc Gly	gca Ala	gga Gly 160	480
tta Leu	act Thr	aca Thr	tct Ser	gtt Val 165	atg Met	gta Val	aaa Lys	aac Asn	gaa Glu 170	aat Asn	tta Leu	aca Thr	aat Asn	ata Ile 175	tca Ser	528
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tct Ser	cca Pro	tat Tyr 195	gta Val	tgt Cys	gca Ala	ggt Gly	att Ile 200	ggc Gly	act Thr	gac Asp	tta Leu	gtg Val 205	tca Ser	gta Val	att Ile	624
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tac Tyr 225	tca Ser	atc Ile	aat Asn	tct Ser	gaa Glu 230	gct Ala	tct Ser	atc Ile	ttt Phe	atc Ile 235	ggt Gly	gga Gly	cat His	ttc Phe	cat His 240	720
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act Thr	tca Ser	aaa Lys	aca Thr 260	gga Gly	ata Ile	tct Ser	aat Asn	cct Pro 265	ggc Gly	ttt Phe	gca Ala	tca Ser	gca Ala 270	aca Thr	ctt Leu	816
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Asn	Pro	Ala 35	Gly	Ser	Val	Tyr	Ile 40	Ser	Ala	Lys	Tyr	Met 45	Pro	Thr	Ala
Ser	His 50	Phe	Gly	Lys	Met	Ser 55	Ile	Lys	Glu	Asp	Ser 60	Lys	Asn	Thr	Gln
Thr 65	Val	Phe	Gly	Leu	Lys 70	Lys	Asp	Trp	Asp	Gly 75	Val	Lys	Thr	Pro	Ser 80
Asp	Ser	Ser	Asn	Thr 85	Asn	Ser	Thr	Ile	Phe 90	Thr	Glu	Lys	Asp	Tyr 95	Ser
Phe	Arg	Tyr	Glu 100	Asn	Asn	Pro	Phe	Leu 105	Gly	Phe	Ala	Gly	Ala 110	Ile	Glλ
Tyr	Ser	Met 115	Asn	Gly	Pro	Arg	11e 120	Glu	Phe	Glu	Val	Ser 125	Tyr	Glu	Thr
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Tyr 145	Cys	Ala	Leu	Asp	Thr 150	Ala	Ala	Gln	Asn	Ser 155	Thr	Asn	Gly	Ala	Gl _y 160
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Ser	Pro	Tyr 195	Val	Cys	Ala	Gly	Ile 200	Gly	Thr	Asp	Leu	Val 205	Ser	Val	Ile
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Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys 115 120 125

Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu 130 135 140

Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val 145 150 155 160

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Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys 180 185 190

Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro 195 200 205

Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro 210 215 220

Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn 225 230 235

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Tyr geg Ala 145	aga Arg 130 gaa Glu	agg Arg tct ser	ttt Phe ctg Leu	gct Ala gca	act Thr gct Ala 150	ttg Leu 135 att Ile	gcg Ala acc Thr	gac Asp cgc Arg	ggg Gly gac Asp	cag Gln gct Ala 155	tac Tyr 140 aac Asn	gca Ala att Ile	aaa Lys act Thr	agt Ser gag Glu	acc Thr 160	
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<210> 11

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<212> DNA

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<211> 864

<212> DNA

<213> Ehrlichia canis

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<210> 13

<211> 399

<212> DNA

<213> Ehrlichia canis

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WO 00/65063

<211> 43

<212> PRT

<213> Ehrlichia chaffeensis

<400> 14

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<400> 15

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Ile Ser Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser 35 40 45

His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly 50 55 60

Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys Val Ile Ser Arg Thr
65 70 75 80

Thr Leu Ser Asp Ile Phe Thr Val Pro Asn Tyr Ser Phe Lys Tyr Glu 85 90 95 Asn Asn Leu Phe Ser Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp 100 105 110

Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Ala Phe Asp Val Lys

Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Tyr Ala Leu 130 135 140

Ser His Leu Leu Gly Thr Glu Thr Gln Ile Asp Gly Ala Gly Ser Ala 145 150 155 160

Ser Val Phe Leu Ile Asn Glu Gly Leu Leu Asp Lys Ser Phe Met Leu 165 170 175

Asn Ala Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr 180 185 190

Ile Cys Ala Gly Ile Gly Ile Asp Leu Val Ser Met Phe Glu Ala Ile 195 200 205

Asn Pro Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Pro Ile 210 215 220

Ser Pro Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile 225 230 235 240

Gly Asn Glu Phe Arg Asp Ile Pro Thr Met Ile Pro Ser Glu Ser Ala 245 250 255

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<212> PRT

<213> Ehrlichia chaffeensis

<400> 16

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Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Pro Val Gln Gly Asp Asn 20 25 30

Ile Ser Gly Asn Phe Tyr Val Ser Gly Lys Tyr Met Pro Ser Ala Ser 35 40 45

His Phe Gly Met Phe Ser Ala Lys Glu Glu Lys Asn Pro Thr Val Ala 50 55 60

Leu 65	Tyr	Gly	Leu	Lys	Gln 70	Asp	Trp	Glu	Gly	Ile 75	Ser	Ser	Ser	Ser	His
Asn	Asp	Asn	Hıs	Phe 85	Asn	Asn	Lys	Gly	Tyr 90	Ser	Phe	Lys	Tyr	Glu 95	Ası
Asn	Pro	Phe	Leu 100	Gly	Phe	Ala	Gly	Ala 105	Ile	Gly	Tyr	Ser	Met 110	Gly	Gly
Pro	Arg	Val 115	Glu	Phe	Glu	Val	Ser 120	Tyr	Glu	Thr	Phe	Asp 125	Val	Lys	Ası
Gln	Gly 130	Asn	Asn	Tyr	Lys	Asn 135	Asp	Ala	His	Arg	Tyr 140	Cys	Ala	Leu	Gly
Gln 145	Gln	Asp	Asn	Ser	Gly 150	Ile	Pro	Lys	Thr	Ser 155	Lys	Tyr	Val	Leu	Le:
Lys	Ser	Glu	Gly	Leu 165	Leu	Asp	Ile	Ser	Phe 170	Met	Leu	Asn	Ala	Cys 175	Ty
Asp	Ile	Ile	Asn 180	Glu	Ser	Ile	Pro	Leu 185	Ser	Pro	Tyr	Ile	Cys 190	Ala	Gly
Val	Gly	Thr 195	Asp	Leu	Ile	Ser	Met 200	Phe	Glu	Ala	Thr	Asn 205	Pro	Lys	Ile
Ser	Tyr 210	Gln	Gly	Lys	Leu	Gly 215	Leu	Ser	Tyr	Ser	Ile 220	Asn	Pro	Glu	Ala
Ser 225	Val	Phe	Ile	Gly	Gly 230	His	Phe	His	Lys	Val 235	Ile	Gly	Asn	Glu	Phe 240
Arg	Asp	Ile	Pro	Thr 245	Leu	Lys	Ala	Phe	Val 250	Thr	Ser	Ser	Ala	Thr 255	Pro
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Ser	Phe	Leu	Pro 20	Gl7.	Ile	Ser	Phe	Ser 25	Asp	Ala	Val	Gìn	Asn 30	Asp	Asr

WO 00/65063

Val Gly Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Val Pro Ser Val Ser 35 40 45

His Phe Gly Val Phe Ser Ala Lys Gln Glu Arg Asn Thr Thr Ile Gly 50 55 60

Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser Thr Ile Ser Lys Asn 65 70 75 80

Ser Pro Glu Asn Thr Phe Asn Val Pro Asn Tyr Ser Phe Lys Tyr Glu 85 90 95

Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Val Gly Tyr Leu Met Asn 100 105 110

Gly Pro Arg Ile Glu Leu Glu Met Ser Tyr Glu Thr Phe Asp Val Lys

Asn Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Lys Tyr Tyr Ala Leu 130 135 140

Thr His Asn Ser Gly Gly Lys Leu Ser Asn Ala Gly Asp Lys Phe Val 145 150 155 160

Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Leu Met Leu Asn Ala 165 170 175

Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys 180 185 190

Ala Gly Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Ile Asn Pro 195 200 205

Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro 210 215 220

Glu Ala Ser Val Phe Val Gly Gly His Phe His Lys Val Ile Gly Asn 225 230 235

Glu Phe Arg Asp Ile Pro Ala Met Ile Pro Ser Thr Ser Thr Leu Thr

Gly Asn His Phe Thr Ile Val Thr Leu Ser Val Cys His Phe Gly Val

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<400> 18

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Ser	Ser	Leu	Pro 20	Gly	Val	Ser	Phe	Ser 25	Asp	Pro	Ala	Gly	Ser 30	Gly	Ile
Asn	Gly	Asn 35	Phe	Tyr	Ile	Ser	Gly 40	Lys	Tyr	Met	Pro	Ser 45	Ala	Ser	His
Phe	Gly 50	Val	Phe	Ser	Ala	Lys 55	Glu	Glu	Arg	Asn	Thr 60	Thr	Val	Gly	Val
Phe 65	Gly	Leu	Lys	Gln	Asn 70	Trp	Asp	Gly	Ser	Ala 75	Ile	Ser	Asn	Ser	Ser 80
Pro	Asn	Asp	Val	Phe 85	Thr	Val	Ser	Asn	Tyr 90	Ser	Phe	Lys	Tyr	Glu 95	Asr
Asn	Pro	Phe	Leu 100	Gly	Phe	Ala	Gly	Ala 105	Ile	Gly	Tyr	Ser	Met 110	Asp	Gl
Pro	Arg	Ile 115	Glu	Leu	Glu	Val	Ser 120	Tyr	Glu	Thr	Phe	Asp 125	Val	Lys	Asr
Gln	Gly 130	Asn	Asn	Tyr	Lys	Asn 135	Glu	Ala	His	Arg	Tyr 140	Cys	Ala	Leu	Sei
His 145	Asn	Ser	Ala	Ala	Asp 150	Met	Ser	Ser	Ala	Ser 155	Asn	Asn	Phe	Val	Phe 160
Leu	Lys	Asn	Glu	Gly 165	Leu	Leu	Asp	Ile	Ser 170	Phe	Met	Leu	Asn	Ala 175	Cys
Tyr	Asp	Val	Val 180	Gly	Glu	Gly	Ile	Pro 185	Phe	Ser	Pro	Tyr	Ile 190	Cys	Ala
Gly	Ile	Gly 195	Thr	Asp	Leu	Val	Ser 200	Met	Phe	Glu	Ala	Thr 205	Asn	Pro	Ly:
Ile	Ser 210	Tyr	Gln	Gly	Lys	Leu 215	Gly	Leu	Ser	Tyr	Ser 220	Ile	Ser	Pro	Gl
Ala 225	Ser	Val	Phe	Ile	Gly 230	Gly	His	Phe	His	Lys 235	Val	Ile	Gly	Asn	Gl:
Phe	Arg	Asp	Ile	Pro 245	Thr	Ile	Ile	Pro	Thr 250	Gly	Ser	Thr	Leu	Ala 255	Gl.
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<213> Ehrlichia canis

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Ile His Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Thr Ala Ser His 35 40 45

Phe Gly Ile Phe Ser Ala Lys Glu Glu Gln Ser Phe Thr Lys Val Leu 50 55 60

Val Gly Leu Asp Gln Arg Leu Ser His Asn Ile Ile Asn Asn Asn Asp 65 70 75 80

Thr Ala Lys Ser Leu Lys Val Gln Asn Tyr Ser Phe Lys Tyr Lys Asn 85 90 95

Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Ile Gly Asn 100 105 110

Ser Arg Ile Glu Leu Glu Val Ser His Glu Ile Phe Asp Thr Lys Asn 115 120 125

Pro Gly Asn Asn Tyr Leu Asn Asp Ser His Lys Tyr Cys Ala Leu Ser 130 135 140

His Gly Ser His Ile Cys Ser Asp Gly Asn Ser Gly Asp Trp Tyr Thr 145 150 155 160

Ala Lys Thr Asp Lys Phe Val Leu Leu Lys Asn Glu Gly Leu Leu Asp 165 170 175

Val Ser Phe Met Leu Asn Ala Cys Tyr Asp Ile Thr Thr Glu Lys Met 180 185 190

Pro Phe Ser Pro Tyr Ile Cys Ala Gly Ile Gly Thr Asp Leu Ile Ser 195 200 205

Met Phe Glu Thr Thr Gln Asn Lys Ile Ser Tyr Gln Gly Lys Leu Gly 210 215 220

Leu Asn Tyr Thr Ile Asn Ser Arg Val Ser Val Phe Ala Gly Gly His 225 230 235 240

Phe His Lys Val Ile Gly Asn Glu Phe Lys Gly Ile Pro Thr Leu Leu 245 250 255

Pro Asp Gly Ser Asn Ile Lys Val Gln Gln Ser Ala Thr Val Thr Leu 260 265 270

Asp Val Cys His Phe Gly Leu Glu Ile Gly Ser Arg Phe Phe 275 285

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<211> 133

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<213> Ehrlichia canis

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Tyr Phe Leu Pro Asn Val Ser Tyr Ser Asn Pro Val Tyr Gly Asn Ser 20 25 30

Met Tyr Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Val Pro

His Phe Gly Ile Phe Ser Ala Glu Glu Glu Lys Lys Lys Thr Thr Val

Val Tyr Gly Leu Lys Glu Asn Trp Ala Gly Asp Ala Ile Ser Ser Gln
65 70 75 80

Ser Pro Asp Asp Asn Phe Thr Ile Arg Asn Tyr Ser Phe Lys Tyr Ala 85 90 95

Ser Asn Lys Phe Leu Gly Phe Ala Val Ala Ile Gly Tyr Ser Ile Gly
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Asn Gln Gly Asn Asn 130

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<213> Ehrlichia canis

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tgtaaaagca tttgccctgc agaattggga ttagtatetg aagcacttgc acaacttgg 300 aataatgcag acaaattaca agtaatttt attacaattg atccaaaaaa tgatactgta 360 gaaaaattaa aagaatttca tgaacatttt gattcaagaa ttcaaatgtt aacaggaaat 420 actgaagaca ttaaaccaata aattaaaaaat tataaaaata atgttggaca agcagataaa 480 gatcatcaaa ttaaccattc tgcaataatg taccttattg acaaaaaagg atcatatctt 540 gttaagcag tccacacttc aaaatcacaa gaaaatcaag tagataagtt actatctta 600 gttaagcag atctgtaaaa tggcat teggaca tuggtaggt ttttttagtg 660 atttttagtg ctaataaca tggcat tggcat teggaca teggataggt ttttttagtg 660

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acagtaaata gteaagattt tttgggaaaa tacatgetag ttttatttgg attttette 240
tgtaaaagea tetgeeetge tgaattagga atageatetg aagtteetee acagettggt 300
aatgacacag acaagttaca agtaatttte attacaattg atecaacaaa tgatactga 360
caaaaattaa aaacatttea tgaacatttt gateetagaa tteaaaatget aacaggeagt 420
geagaagata ttgaaaaaat aataaaaaat tacaaaatat atgttggaca ageagataaa 480
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WO 00/65063

21

Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Thr Lys His His Asp Thr Pro Asn Thr Thr Ile Pro Asn Glu Asp Gly Ile 4.0 Gln Ser Ser Phe Ser Leu Ile Asn Gln Asp Gly Lys Thr Val Thr Ser 55 Gln Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ala Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Leu Val Ser Glu Ala Leu 90 Ala Gin Leu Gly Asn Asn Ala Asp Lys Leu Gln Val Ile Phe Ile Thr 105 100 Ile Asp Pro Lys Asn Asp Thr Val Glu Lys Leu Lys Glu Phe His Glu 120 His Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Thr Glu Asp Ile 130 Asn Gln Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys 155 Asp His Gln Ile Asn His Ser Ala Ile Met Tyr Leu Ile Asp Lys Lys 170 165 Gly Ser Tyr Leu Ser His Phe Ile Pro Asp Leu Lys Ser Gln Glu Asn 180 Gln Val Asp Lys Leu Leu Ser Leu Val Lys Gln Tyr Leu 200 195 <210> 24 <211> 205 <212> PRT <213> Ehrlichia chaffeensis <400> 24 Met Lys Val Ile Lys Phe Ile Leu Asn Ile Cys Leu Leu Phe Ala Ala Ile Phe Leu Gly Tyr Ser Tyr Val Thr Lys Gln Gly Ile Phe Gln Val Arg Asp His Asn Thr Pro Asn Thr Asn Ile Ser Asn Lys Ala Ser Ile 40

Thr Thr Ser Phe Ser Leu Val Asn Gln Asp Gly Asn Thr Val Asn Ser

65	Asp	Phe	Leu	Gly	Lys 70	Tyr	Met	Leu	Val	Leu 75	Phe	Gly	Phe	Ser	Ser 80	
Cys	Lys	Ser	Ile	Cys 85	Pro	Ala	Glu	Leu	Gly 90	Ile	Ala	Ser	Glu	Val 95	Leu	
Ser	Gln	Leu	Gly 100	Asn	Asp	Thr	Asp	Lys 105	Leu	Gln	Val	Ile	Phe 110	Ile	Thr	
Ile	Asp	Pro 115	Thr	Asn	Asp	Thr	Val 120	Gln	Lys	Leu	Lys	Thr 125	Phe	His	Glu	
His	Phe 130	Asp	Pro	Arg	Ile	Gln 135	Met	Leu	Thr	Gly	Ser 140	Ala	Glu	Asp	Ile	
Glu 145	Lys	Ile	Ile	Lys	Asn 150	Tyr	Lys	Ile	Tyr	Val 155	Gly	Gln	Ala	Asp	Lys 160	
Asp	Asn	Gln	Ile	Asp 165	His	Ser	Ala	Ile	Met 170	Tyr	Ile	Ile	Asp	Lys 175	Lys	
Glγ	Glu	Tyr	Ile 180	Ser	His	Phe	Ser	Pro 185	Asp	Leu	Lys	Ser	Thr 190	Glu	Asn	
Gln	Val	Asp 195	Lys	Leu	Leu	Ser	Ile 200	Ile	Lys	Gln	Tyr	Leu 205				
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WO 00/65063

Lys 65	Asp	Phe	Leu	Gly	Lys 70	His	Met	Leu	Val	Leu 75	P'ne	Gly	Phe	Ser	Ser 80	
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gat Asp	caa Gln	ctt Leu	ggc Gly 100	aac Asn	gaa Glu	tct Ser	gac Asp	aag Lys 105	tta Leu	caa Gln	gta Val	gtc Val	ttt Phe 110	ata Ile	act Thr	336
att Ile	gat Asp	cca Pro 115	aca Thr	aaa Lys	gat Asp	act Thr	gta Val 120	gaa Glu	aca Thr	cta Leu	aaa Lys	gag Glu 125	ttt Phe	cac	aaa Lys	384
aat Asn	ttt Phe 130	gac Asp	tca Ser	cgg Arg	att Ile	caa Gln 135	atg Met	tta Leu	aca Thr	gga Gly	aac Asn 140	att Ile	gaa Glu	gct Ala	att Ile	432
aat Asn 145	caa Glr.	ata Ile	gta Val	caa Gln	999 Gly 150	tac Tyr	aaa Lys	gta Val	tat Tyr	gta Val 155	ggt Gly	cag Glr	cca Pro	gac Asp	aat Asn 160	480
gat Asp	aac Asn	caa Gln	att Ile	aac Asn 165	cat His	tct Ser	gga Gly	ata Ile	atg Met 170	tat Tyr	att Ile	gta Val	gac Asp	aag Lys 175	aaa Lys	528
gga Gly	gaa Glu	tat Tyr	tta Leu 180	aca Thr	cat His	ttt Phe	gta Val	cca Pro 185	gat Asp	tta Leu	aag Lys	tca Ser	aaa Lys 190	gag Glu	cct Pro	576
caa Gln	gtg Val	gat Asp 195	aa a Lys	tta Leu	ctt Leu	tct Ser	tta Leu 200	att Ile	aag Lys	cag Gln	tat Tyr	ctt Leu 205	taa			618
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Ile	Phe	Leu	Gly 20	Tyr	Ser	Тут	Ile	Thr 25	Lys	Gln	Gly	Ile	Phe 30	Gln	Pro	
Lys	Leu	His 35	Asp	Ser	Pro	Asp	Val 40	Asn	Ile	Ser	Asn	Lys 45	Ala	Asp	Ile	
Asn	Thr 50	Ser	Phe	Ser	Leu	Tle 55	Asn	Gln	Asp	Gly	Ile 60	Thr	Ile	Ser	Ser	

Lys 65	Asp	Phe	Leu	Gly.	Lys 70	His	Met	Leu	Val	Leu 75	Phe	GIÀ	Phe	Ser	ser 80	
Cys	Lys	Thr	Ile	Cys 85	Pro	Met	Glu	Leu	Gly 90	Leu	Ala	Ser	Thr	Ile 95	Leu	
Asp	Glr.	Leu	Gly 100	Asn	Glu	Ser	Asp	Lys 105	Leu	Gln	Val	Val	Phe 110	Ile	Thr	
Ile	Asp	Prc 115	Thr	Lys	Asp	Thr	Val 120	Glu	Thr	Leu	Lys	Glu 125	Phe	His	Lys	
Asn	Phe 130	Asp	Ser	Arg	Ile	Gln 135	Met	Leu	Thr	Gly	Asn 140	Ile	Glu	Ala	Ile	
145					150		Lys			155					160	
				165			Gly		170					175		
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Gln	Val	Asp 195	Lys	Leu	Leu	Ser	Leu 200	Ile	Lys	Gln	Tyr	Leu 205				
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tta Leu	att Ile	ggt Gly 35	act Thr	ggt	tct Ser	atg Met	act Thr 40	gga Gly	gta Val	tat Tyr	tat Tyr	cct Pro 45	ata Ile	gga Gly	ggt Gly	14
agc Ser	ata Ile 50	tgt Cys	agg Arg	ttt Phe	att Ile	gca Ala 55	tct Ser	gat Asp	tat Tyr	ggt Gly	aat Asn 60	gat Asp	aat Asn	aac Asn	agc Ser	19

WO 00/65063 PCT/US00/10886

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tct Ser	atg Met	cgt Arg	tat Tyr	gca Ala 85	aat Asn	atg Met	gat Asp	ata Ile	ggt Gly 90	att Ile	att Ile	caa Gln	tct Ser	gat Asp 95	tta Leu	288
gag Glu	tac Tyr	tat Tyr	gca Ala 100	tat Tyr	aat Asn	ggt Gly	att Ile	ggt Gly 105	tta Leu	tat Tyr	gaa Glu	aaa Lys	atg Met 110	cca Pro	gca Ala	336
atg Met	agg Arg	cat His 115	cta Leu	aga Arg	ata Ile	tta Leu	tct Ser 120	tca Ser	tta Leu	cat	aaa Lys	gaa Glu 125	tat Tyr	ctt Leu	aca Thr	384
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ggc Gly 145	aaa Lys	aga Arg	gtt Val	aat Asn	att Ile 150	ggt Gly	agt Ser	cct Pro	ggt Gly	act Thr 155	ggt Gly	gta Val	aga Arg	ata Ile	gca Ala 160	480
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gat Asp 225	gat Asp	ctc Leu	ata Ile	gat Asp	aaa Lys 230	tta Leu	cat Hıs	act Thr	aag Lys	tat Tyr 235	acc Pro	tat Tyr	tat Tyr	aaa Lys	agg Arg 240	720
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gtt Val	tca Ser	gta Val	aaa Lys 260	gct Ala	tct Ser	tta Leu	ata Ile	aca Thr 265	act Thr	act	gaa Glu	tta Leu	agc Ser 270	aat Asn	gag Glu	816
ttg Leu	gcc Ala	tat Tyr 275	aaa Lys	gtt Val	gtt Val	aaa Lys	tot Ser 280	ttg Leu	gtt Val	agc Ser	cat His	tta Leu 285	cat His	gaa Glu	cta Leu	86≟

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пі	S G1 29		.e 11:	ır Gı	y Ale	а ьег 29!		g As:	n Le	u Th	r Val 300		s Ası	o Me	t Val
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	00 > 2		s Tla	a I.e.1	. Val	Thr	Dha	I All		W- 1	Val	7 00	77-7	Dh -	Q
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ASI	HIC	L Alc	2 116		ser	inr	Asp	Ser 25		. GIU	Asp	Lys	Gln 30	Tyr	Ile
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	_	115		٥			120				-,-	125	*1*		****
Ile	Va l	Val	Ara	Δla	Aen	Ser	Δεη	Tla	Ser	Vell	Ile	7.55	7. ~~	Tl ~	T ~
	130		9	2.1C4	~	135	17011	* T C	267	val	140	Asp	Asp	тте	ьуs
C1	T	70 -	•••	-	~ 7	~ 3	_								
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Val Met Ala Glu Leu Lys Ser Ser Glu Gln Ala Gln Ala Leu Cys Asp

WO 00/65063 PCT/US00/10886

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3 l

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WO 00/65063 PCT/US00/10886

33

Glu Leu Gly Ser Asp Gln Glu Val Ile Val Ser Glu Gly Leu Ile Glu 115 120 125

His Thr His Ser Asp Leu Ser Phe Asn Ala Ile Ile Ala Lys Ile Ile 130 135 140

Asp Ser Leu Ile Lys 145

Intern val Application No PCT/US 00/10886

A. CLASSIFICATION OF SUBJECT MATTER
I PC 7 C12N15/31 C07K14/29 A61K39/02 C12Q1/68 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) BIOSIS C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ° Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. WO 98 16554 A (UNIV FLORIDA) 23 April 1998 (1998-04-23) Χ 1 - 24the whole document BOWIE MICHAEL V ET AL: "Potential value Χ 1-4, of major antigenic protein 2 for 7-13, serological diagnosis of heartwater and 21-24 related Ehrlichial infections." CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, vol. 6, no. 2, March 1999 (1999-03), pages 209-215, XP000939015 ISSN: 1071-412X the whole document X Further documents are listed in the continuation of box C. IX Patent family members are listed in annex. Special categories of cited documents: T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-"O" document referring to an oral disclosure, use, exhibition or other means ments, such combination being obvious to a person skilled in the art. * document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 2 2 12 2000 5 September 2000 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 ANDRES S.M.

Form PCT/ISA/210 (second sheet) (July 1992)

Intern al Application No PCT/US 00/10886

	
ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
NYIKA A ET AL.: "A DNA vaccine protects mice against the rickettsial agent Cowdria ruminantium." PARASITE IMMUNOLOGY (OXFORD), vol. 20, no. 3, March 1998 (1998-03), pages 111-119, XP000939081 ISSN: 0141-9838 the whole document	1-4, 6-14, 17-19
MAHAN S M ET AL: "Molecular cloning of a gene encoding the immunogenic 21 kDa protein of Cowdria ruminantium." MICROBIOLOGY (READING), vol. 140, no. 8, 1994, pages 2135-2142, XP000939016 the whole document	1-4, 7-13, 21-24
	NYIKA A ET AL: "A DNA vaccine protects mice against the rickettsial agent Cowdria ruminantium." PARASITE IMMUNOLOGY (OXFORD), vol. 20, no. 3, March 1998 (1998-03), pages 111-119, XP000939081 ISSN: 0141-9838 the whole document MAHAN S M ET AL: "Molecular cloning of a gene encoding the immunogenic 21 kDa protein of Cowdria ruminantium." MICROBIOLOGY (READING), vol. 140, no. 8, 1994, pages 2135-2142, XP000939016

International application No. PCT/US 00/10886

Box	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION sheet PCT/ISA/210
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	rnational Searching Authority found multiple inventions in this international application, as follows:
	see additional sheet
1.	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. X	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark o	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 10 to 19 are directed to a method of treatment of the human/animal body, and claim 20 (as far as an in vivo method is concerned) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

- 1. Claims: 1-24
 - 1.1. Claims: 1-2,6-7,10-11,17-19,21-22 (all partially)
 A composition comprising a polynucleotide encoding an antigen from Rickettsia spp. and methods for using it in protection of a host against a disease or death, or in diagnostic.
 - 1.2. Claims: 1-4,6-13,17-24 (all partially), and claims 5, 15 (totally)

Compositions comprising SEQ IDs 3,4; 7,14; 8,15; 9,16; 10,17; 11,18 and 22,24 (corresponding to the MAP1, VSA1 to VSA5 and MAP2 antigens from Ehrlichia chaffeensis) and methods for using them in protection of a host against a disease or death, or in diagnostic.

- 1.3. Claims: 1-4,6-13,17-24 (all partially)
 Compositions comprising SEQ IDs 12,19; 13,20 and 21,23
 (corresponding to the VSA1, VSA2 and MAP2 antigens
 from Ehrlichia canis) and methods for using them in
 protection of a host against a disease or death, or in
 diagnostic.
- 1.4. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 16 (totally)

A compositions comprising SEQ IDs 4 and 5 (corresponding to the MSP-4 antigen from Anaplasma marginale) and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.5. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 14 (totally)

Compositions comprising SEQ IDs 1,2 and 25,26 (corresponding to the antigens MAP1 and MAP2 from Cowdria ruminantium) and methods for using them in protection of a host against a disease or death, or in diagnostic.

2. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 27 and 28 (corresponding to the 1hworf3 antigen from Cowdria ruminantium) and methods

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

for using it in protection of a host against a disease or death, or in diagnostic.

3. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 29 and 30 (corresponding to the 4hworfl antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

4. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 31 and 32 (corresponding to the 18hworfl antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

5. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 33 and 34 (corresponding to the 3gdorf3 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

ormation on patent family members

Interr ial Application No
PCT/US 00/10886

Patent document cited in search report		Publication date		atent family nember(s)	Publication date
WO 9816554	A	23-04-1998	US AU ZA	6025338 A 4913097 A 9709320 A	15-02-2000 11-05-1998 16-03-1999

Form PCT/ISA/210 (patent tarnity annex) (July 1992)